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QUANTITATIVE DETERMINATION OF THE BALANTIDICIDAL ACTIVITY OF CERTAIN DRUGS AND CHEMICALS AS A BASIS FOR TREATMENT OF INFECTIONS WITH BALANTIDIUM COLI

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Infections with *Balantidium coli* are much more prevalent in the Philippine Islands than has been generally recognized. The first case reported was that by Strong (1901). In Bilibid Prison 10 cases were found in the routine examination of stools during 1911, and 11 cases in 1912. At the Philippine General Hospital 8 cases have been observed during 1911 and 1912.

Balantidiasis is characterized by the frequency of latent infections, infections in which the patient may show no clinical symptoms, or only occasional attacks of diarrhœa, over long periods of time. Of the 29 cases observed in Bilibid Prison and at the Philippine General Hospital during 1911 and 1912 only 5 have exhibited diarrhœal or dysenteric symptoms. Bowman (1909 and 1911) published a description of 3 fatal cases in Manila which came to necropsy. A further characteristic of these infections is that the parasites only appear in the stools of the patient at irregular intervals. On account of these peculiarities of this disease and the parasite, infections are probably frequently overlooked in the routine examination of stools.

The medical importance of *Balantidium coli*, notwithstanding these conditions, consists in the facts that persons parasitized

with this protozoön are likely sooner or later to develop balantidial dysentery; that, once the dysenteric condition is established, it is apt to run a fatal course; and in that no efficient treatment is known for this disease.

Various drugs and chemicals given by mouth or used as enemata have been employed in the treatment of balantidial infections. Authors have reported the disappearance of the balantidia from the stools and the improvement of their patient following treatment with certain of these substances; but the specific therapeutic action of them has in each case been rendered doubtful by the fact that other authors have found the same treatments worthless. The explanation of these inconsistent results is to be found in the latency characteristic of the disease and in the tendency of the parasites to disappear spontaneously from the stools of infected persons for variable lengths of time. As a result of these peculiarities of the infection, the determination of the efficiency of any treatment of balantidiasis by clinical observation is extremely fallacious unless the patient be kept under observation for a long time.

It has, therefore, seemed possible that the probable value of different drugs and chemicals in the treatment of this infection could be more quickly and accurately determined by laboratory tests of the balantidicidal action of them *in vitro*.

The practical value of tests *in vitro* of the action of drugs and chemicals on parasitic protozoa has been demonstrated by the investigations of Vedder (1911 and 1912) and Rogers (1912a and 1912b) in the application of ipecac and its alkaloid, emetine, to the treatment of entamœbic dysentery. Vedder found that cultures of amœbæ were killed by solutions of different fluid extracts of ipecac in dilutions of 1 in 10,000 to 1 in 50,000 and that the active amœbicidal principle of ipecac was probably emetine, since a solution of this latter substance killed the amœbæ in dilutions of 1 in 100,000. These tests established a scientific basis for the empiric treatment of amœbic dysentery with ipecac. Very recently Rogers, undoubtedly influenced by Vedder's investigation, has tested *in vitro* the entamœbicidal properties of the soluble salts of emetine. He found that *Entamœba histolytica* was killed by dilutions of 1 in 100,000 of this alkaloid. Applying these laboratory results to clinical medicine, Rogers has reported results which are so striking in the treatment of entamœbic dysentery and liver abscesses that, if substantiated, they will prove that we have a specific for this disease.

The conditions for testing the balantidicidal action of drugs and chemicals *in vitro* are extremely favorable. *Balantidium coli,* under favorable conditions, remains alive and active in the fæces for several days. Moreover, we have, in the active locomotion and the persistent activity of the oral cilia during life, and in the disintegration of the protozoön at death, the most delicate and at the same time the most striking indicators of injury to, and death of, the parasite. The active movements of *Balantidium coli* usually persist up to, and the movement of the oral cilia even after, the beginning of disintegration of the protozoön. Death of the parasite is usually accompanied or closely followed by disintegration. This disintegration takes place by the extrusion of the protoplasm through the oral, and sometimes also the anal, orifice of the protozoön. The process of protoplasmic extrusion is closely correlated with the balantidicidal activity of the substance employed. In slightly toxic solutions the balantidia exhibit only the protrusion of small buds of protoplasm, the organism still maintaining its active movements. In more toxic solutions the extrusion of the protoplasm becomes progressively greater until the protozoön collapses. And in eminently toxic solutions this disintegration becomes explosive in character, the ectosarc being ruptured and the whole contents liberated at once. An exception to this disintegration of balantidium occurs in the presence of certain chemicals, such as the salts of mercury, which act as a fixative. In this case the protozoön is killed and fixed with cilia extended.

There exist records of a few balantidicidal tests performed *in vitro,* but these tests have been limited to acids, alkalies, alcohol, and a few common salts, and definite quantitative determinations of the balantidicidal limits of dilution of the substances have not been made. Thus Glaessner (1908) made the following crude tests of the action of a few chemicals on *Balantidium coli:*

Experiment.	Result after 6 hours.
1. Ten grams of fæces were added to 40 cubic centimeters of water at 40° C.	Balantidia mostly dead, a few were living.
2. Ten grams of fæces were added to 40 cubic centimeters of 0.6 per cent NaCl.	Some of the balantidia dead.
3. Ten grams of fæces were added to 40 cubic centimeters of 1 per cent alcohol.	Balantidia dead.
4. Ten grams of fæces were added to 40 cubic centimeters of 5 per cent alcohol.	Balantidia dead.
5. Ten grams of fæces were added to 40 cubic centimeters of 0.3 per cent NaCl.	Balantidia dead.

Experiment.	Result after 6 hours.
6. Ten grams of fæces were added to 40 cubic centimeters of 0.4 per cent solution of caustic soda.	Balantidia living and motile.
7. Ten grams of fæces were added to 40 cubic centimeters of 0.2 per cent acetic acid.	Balantidia dead.
	Result after 5 minutes.
8. Ten grams of fæces were added to 40 grams of 1 per cent mercuric chloride.	Balantidia dead.
9. Ten grams of fæces were added to 40 grams NaCl (0.8 per cent) cooled to 5° C.	Balantidia dead.

Ortman (1891) observed the action of certain chemicals on balantidia in hanging-drop preparations as follows:

Chemical.	Strength of solution.	Time of exposure.	Result.
		H. mi.	
NaCl	¾ per cent	0 45	Balantidia dead.
Karlsbader water	----------	----------	Negative.
Potassium permanganate	1:3,000	0 30	Balantidia dead.
Hydrochloric acid	2:2,000	0 20	Do.
Do	1:3,000	0 13	Do.
Acetic acid	1:1,000	0 20	Some of the balantidia living.
Tannic acid	1: 400	0 25	Do.
Do	1: 200	0 11	Balantidia dead.
Do	1: 100	0 3	Do.
Quinine sulphate	1:2,000	2 30	Do.
Do	1:1,000	0 5	Do.

In the experiments described in this paper, an attempt has been made to determine quantitatively the balantidicidal action; first, of those drugs and chemicals which have been more or less successfully employed in the treatment of other protozoan infections, and, if these failed to be active; secondly, to seek for some efficient balantidicidal drug or chemical that could be used in the treatment of balantidial dysentery. The first of these purposes has been limited to some extent by my inability to procure in Manila all of the drugs and chemicals that have been employed in the treatment of protozoan diseases. However, a certain number of them have been obtainable which represent the chief groups of these substances, as the aniline dyes, the arsenic and the antimony compounds, ipecac and its alkaloid, emetine, and the salts of quinine. The second purpose, to seek some new drugs or chemicals having a specific action on *Balantidium coli*, has also proceeded according to a definite plan which will be apparent later.

Balantidium coli has been found to be extremely sensitive to changes in the tonicity of the medium in which it is placed. It was, therefore, necessary to determine the most favorable tonicity of the fluid to be employed in making the dilutions of the drugs and chemicals in order not to introduce a source of error from this factor in the tests. It was found that 0.85 per cent normal physiological salt solution was slightly hypertonic, while distilled water was slightly hypotonic, for *Balantidium coli.* Therefore, comparative tests were made with different strengths of sodium chloride solutions, and it was decided that 0.5 per cent solution represented about the optimum tonicity for this organism. A departure from the use of 0.5 per cent sodium chloride for making the dilutions was found to be necessary in certain cases. Thus, in the case of mercuric iodide, which is not soluble in water or physiological salt solution, a 2 per cent solution of potassium iodide in water was employed for dissolving and making the first dilution of 1 in 50; the subsequent dilutions were then made with 0.5 per cent sodium chloride solution. In this case the first and consequently the subsequent dilutions contained the same percentage of potassium iodide as of mercuric iodide. A series was then run with potassium iodide as a control, which was found to be inert. Again, in testing some of the compounds of silver, which are precipitated by weak solutions of sodium chloride, the dilutions were made with distilled water and a control run with distilled water.

In the preliminary tests of each substance dilutions of 1 in 50, 1 in 500, 1 in 5,000, and 1 in 50,000 were made. These, when mixed with equal parts of the fluid fæces containing the balantidia, gave final dilutions of 1 in 100, 1 in 1,000, 1 in 10,000, and 1 in 100,000. The limits of the balantidicidal action of the substance between any two of these dilutions having been determined, the more precise limit of its action was then determined by making intermediate dilutions between the highest positive and the lowest negative dilutions.

Dilutions of the substances to be tested were made in the following manner. If, as in most cases, the substance was a solid, 0.2 gram was weighed out with an analytical balance in a 10 cubic centimeters volumetric flask. The substance was then dissolved with 0.5 per cent sodium chloride solution and the flask filled to the graduation mark. This gave a dilution of 1 in 50. If, as was rarely the case, the substance was a

liquid, the first dilution was made by measuring 0.2 cubic centimeter of it and making up to 10 cubic centimeters with the salt solution in the volumetric flask as before. This small quantity of the drug or chemical was used throughout in making the primary dilutions because some of the substances were obtainable only in small quantities. The succeeding dilutions were made in 50 cubic centimeters volumetric flasks by measuring appropriate amounts from each preceding dilution of the substance with a graduated pipette and filling the flask to the graduation mark with the diluting liquid.

The balantidia used in making these tests were in part derived from men, in part from domesticated pigs, and in part from a monkey infected from a pig. *Balantidium coli suis* is generally considered to be identical with *Balantidium coli hominis;* it has been found possible to infect monkeys with the balantidia from the pig; and comparative tests of the same drug or chemical made with the pig and the human balantidia have given uniform results. Balantidia from the pig and monkey have been employed in part in these tests, because human cases of balantidiasis which showed a sufficient number of the organisms in the stools could not at all times be obtained. The material used has consisted of fresh fæces containing living and actively motile balantidia. Unless the fæces was sufficiently fluid, a portion of it was rubbed up in 0.5 per cent sodium chloride solution to fluid consistency.

A large platinum loopful of the fluid fæces containing the balantidia was placed on a microscope slide. Beside it was then placed a drop of equal size of the dilution of the substance to be tested. The two drops were then thoroughly mixed and a cover-glass placed upon it. The edges of the cover-glass were then sealed with vaseline to prevent evaporation. Similar preparations were made of all of the dilutions of the drug or chemical being tested and of a control, consisting of a drop of the fæces and a drop of 0.5 per cent sodium chloride or, in case distilled water had been used as a diluting fluid, distilled water. These preparations were observed with low magnification under the microscope at frequent intervals, and the effect on the balantidia noted. The intervals of observation were usually one, two, five, ten, fifteen, thirty, and sixty minutes. In most cases the observations did not extend beyond one hour, since it was considered that a substance at a given dilution is of little value if it did not kill the organisms within that

period of time. Indeed, if the balantidicidal action was not instantaneous or did not take place within a few minutes, the substance at the given dilution has not been considered to be of practical value.

As an example of these tests the following one of silver nitrate is quoted from my notes:

TESTS OF THE BALANTIDICIDAL ACTION OF SILVER NITRATE ON "BALANTIDIUM COLI SUIS"

Control preparation made at 9.15 a. m. Balantidia active. 10.15, balantidia active. 11.10, balantidia active.

Dilution of 1: 100 made at 9.17 a. m. Balantidia killed and fixed immediately.

Dilution of 1: 1000 made at 9.20 a. m. Locomotion and movement of cilia of the balantidia lost immediately. 9.21, extrusion of the contents of the balantidia.

Dilution of 1: 10,000 made at 9.35 a. m. Balantidia more or less actively motile; 9.35½, locomotion of all balantidia have ceased; 9.36, balantidia have collapsed and contents extruded.

Dilution of 1: 20,000 made at 10.09 a. m. Balantidia sluggishly motile; 10.09½, balantidia motionless, oral cilia motile; 10.10, the same; 10.11, some of the balantidia show extrusion of small drops of protoplasm from the cytostome; 10.12, more pronounced and widespread extrusion of the protoplasm from both the cytostome and cytopyge of the balantidia; 10.15, progressive extrusion of the protoplasm of the balantidia; 10.16, the same; 10.20, the same; 10.30, all balantidia have disintegrated.

Dilution of 1: 25,000 made at 10.30½ a. m., balantidia actively motile; 10.42, the same; 10.44, some of the balantidia are nearly motionless with drops of protoplasm extruding from the cytostome and cytopyge; 10.48, balantidia sluggishly motile with large buds of protoplasm extruding from the oral and anal orifices; 10.53, the same; 11.06, some of the balantidia still motile; 11.39, the same.

Dilution of 1: 100,000 made at 9.43½ a. m. 9.43, balantidia actively motile; 9.45, the same; 9.52, the same; 10.00, the same; 10.34 the same; 11.10, the same.

From these tests it may be concluded that silver nitrate is balantidicidal to a dilution lying between 1 in 20,000 and 1 in 25,000.

The results of the first series of tests of the balantidicidal action of drugs and chemicals are given in Table I. In this table are not included all of the experiments made nor all of the dilutions tested in many of the experiments; only one experiment with each substance and the critical dilutions which determine quantitatively the balantidicidal activity of the substance are given.

TABLE I.—*The balantidicidal action of various chemicals.*

Substance tested.	Balantidicidal action. Dilution. 1:100.	1:200.	1:500.	1:600.	1:800.	1:1,000.	1:10,000.	1:20,000.	1:25,000.	1:100,000.	Control.
Atoxyl	0	------	------	------	----	0	0	------	----	0	0
Sodium arsenate	0	------	------	------	----	0	0	------	----	0	0
Antimonyl potassium tartrate	0	------	------	------	----	0	0	------	----	0	0
Trypan red	0	------	------	------	----	0	0	------	----	0	0
Methylene blue (medicinal)	±	------	------	------	----	0	0	------	----	0	0
Fluid extract of ipecac	±	±	0	------	----	0	0	------	----	0	0
Emetine hydrochloride	±	------	0	------	----	0	0	------	----	0	0
Quinine hydrochlorate	+ (5–10 min.)	+ (10–15 min.)	+ (60 min.)	+ (60 min.)	0	0	0	------	----	0	0
Copper sulphate	+	------	------	------	----	+ (30 min.)	0	------	----	0	0
Mercuric chloride	+	------	------	------	----	+	+	+ (1 min.)	----	0	0
Mercuric iodide	+	------	------	------	----	+	+	+ (instantly)	0	0	0
Silver nitrate	+	------	------	------	----	+	+ (instantly)	+ (2–15 min.)	±	0	0

This series of tests presents some interesting and unexpected results.

In the first place, it is noteworthy that the compounds of arsenic and antimony and the aniline dyes, which have been so extensively employed with more or less success in the treatment of other protozoan diseases, especially the trypanosomiases, have proved to possess little or no balantidicidal action. Atoxyl, sodium arsenate, antimonyl potassium tartrate, and trypan red are absolutely inert in the low dilution of 1 part in 100 after acting for one hour on *Balantidium coli*.[1] Medicinal methylene blue is scarcely more active, a part only of the balantidia being killed after exposure for one hour to a dilution of 1 part in 100 of this dye.

[1] However, the fact should not be overlooked that some of these substances may be more balantidicidal *in vivo* than *in vitro*. Such has been found to be the case with certain arsenic compounds in the treatment of trypanosomiasis.

The second surprising result is the feeble balantidicidal action of ipecac and its alkaloid, emetine, which are used so successfully in the treatment of entamœbic dysentery. In the low dilutions of 1 part in 100 and 1 part in 200, ipecac shows only feeble balantidicidal action, more or less but not all of the protozoa being killed after exposure to its action for one hour. Emetine hydrochloride, which has been proved by Vedder and Rogers to possess specific entamœbacidal properties to an eminent degree, proves to be scarcely as balantidicidal as ipecac.

Quinine, which is a specific for malaria and which has been frequently employed in the treatment of balantidiasis, is somewhat more active. The hydrochloride has killed all of the balantidia in the dilution of 1 part in 100 in five to ten minutes, in the dilution of 1 part in 300 in fifteen to thirty minutes, in the dilution of 1 part in 400 in thirty to sixty minutes, and in the dilutions of 1 part in 500 and 1 part in 600 in sixty minutes. In dilutions of 1 part in 800 and higher, it is inactive. The relatively feeble balantidicidal action of this drug does not promise much success in its application to the treatment of balantidiasis.

These well-known protozoicidal substances having been proved to possess feeble or no balantidicidal action, attention was turned to the salts of the heavy metals.

Copper sulphate, as is well known, displays a profound toxic action toward certain of the lower organisms. It is said to kill fresh-water algæ in the remarkably high dilution of 1 part in 1,000,000, and it has consequently been widely recommended for the purification of water supplies. Its balantidicidal strength, however, has proved to be only moderate. In the dilution of 1 part in 1,000 it killed all of the balantidia in thirty minutes. In higher dilutions it was inert.

The salts of mercury possess eminent germicidal properties, and they have been extensively employed not only as antiseptics and disinfectants, but in the treatment of spirochæte and protozoan infections. The experiments with the chloride and the iodide of mercury have demonstrated that they are also strongly balantidicidal. In dilution as high as 1 part in 20,000 they kill all of the balantidia within one minute.

Silver is said to be one of the most toxic metals for bacteria and protozoa, but unlike mercury to be comparativey innoxious for the mammalian organism. Tests of the balanti-

dicidal action of silver nitrate show it to be an equally efficient balantidicide as the more poisonous mercurial salts. It does not act quite so quickly at the dilution of 1 part in 20,000, but its balantidicidal activity extends to slightly higher dilutions, a part of the balantidia being killed at the dilution of 1 part in 25,000.

The salts of mercury and silver are, therefore, much more balantidicidal than any of the other drugs or chemicals that have been tested; but their application to the treatment of balantidiasis is subject to certain limitations. The salts of mercury and silver nitrate are precipitated on coming in contact with albumin, and the albumin is coagulated, forming an impervious layer. Therefore, when employed for local treatment they are quickly rendered inert and possess little power of penetrating the tissues.

A search was, therefore, instituted for some compound of these metals which possess to some degree the eminent balantidicidal activity, which are not precipitated by albumin, and which might at the same time be less toxic than the ordinary inorganic salts. Substances satisfying these requirements to a greater or lesser degree appear to exist in the organic compounds of silver. Some 15 or 20 of these compounds of silver are known. They are said not to be precipitated by albumin or, if precipitated, to form compounds soluble in an excess of albumin. And it is claimed for many of them that they are as strongly germicidal as, and much less toxic for man than, the nitrate of silver. Accordingly, as many of the more promising of them as could be obtained have been tested for their balantidicidal value. The name, composition, percentage of metallic silver, and notes on the toxicity for mammals of the compounds, and the results of the tests of their balantidicidal action are given in Table II. Corresponding data of silver nitrate are included for comparison.

It is evident from this table that these organic compounds of silver are extremely variable in their balantidicidal action. In general, the larger the percentage of metallic silver, the more actively balantidicidal is the compound. But this does not invariably hold good; for argyrol, which contains about one-third as much silver, is only one two-hundredth as balantidicidal as silver nitrate. It is probable that a number of factors influence the balantidicidal action of these compounds. Of these, the silver content is perhaps the chief; but the action on albumin

and the readiness with which the compound is reduced to metallic silver probably play a part in the process. Among these silver compounds several, especially ichtargan and actol, look promising for the treatment of balantidiasis. These compounds are relatively nontoxic; while they are precipitated by albumin, they are said to form soluble compounds; and they possess a balantidicidal activity which is as great, in proportion to the silver contained, as silver nitrate.

In the application of the organic compounds of silver to the treatment of balantidiasis, there are four ways in which they might be employed, namely: first, by subcutaneous or intravenous injections; second, by high rectal enemata; third, by mouth; and, fourth, by appendicostomy and colon irrigations. The first of these methods, by subcutaneous or intraveneous injections, has a precedent in Roger's treatment of entamœbic dysentery by subcutaneous injections of the soluble salts of emetine. In such treatment, 1 part of the silver compound to the number of parts of the body weight which are equivalent to the highest efficient balantidicidal dilution of the compound would have to be administered. Whether such a dose could be safely given would have to be determined experimentally. It is noteworthy in this connection that for several of these compounds such a dose has been experimentally determined to be harmless for guinea pigs (Table II). The chief objection to rectal enemata is that they cannot be made high enough to reach all of the infected gut. Nevertheless, it is one of the most convenient methods of treatment, and, if the large intestine be first emptied of fæcal matter as completely as possible, should be efficient so far as the infected areas can be reached. For this method of treatment, solutions of the compounds of silver of a strength at least equivalent to the highest efficient balantidicidal dilution should be employed. Capsules coated with creatin, salol, or other substance that would be dissolved only just before discharge into the large intestine would probably be an efficient method of treatment, since by this means the whole length of the large intestine could be reached with the balantidicidal agent. In cases of balantidial infection in which dysenteric symptoms are established and which yield to none of the other methods of treatment, appendicostomy and the flushing out of the whole large intestine with the balantidicidal solution would undoubtedly be the most efficient method of treatment and would under the circumstances be advisable.

TABLE II.—*Composition and action of various silver compounds.*

Silver compound.	Composition.	Percentage of silver contained in compound.	Reaction with albumin.	Toxicity for mammals.	Balantidicidal action. Dilution. 1:100.	1:200.	1:500.	1:600.	1:800.	1:1,000.	1:10,000.	1:15,000.	1:20,000.	1:25,000.	1:100,000.	Control.
Argyrol	Silver and vitellin	20 to 25		1 part to 10,000 of body weight given subcutaneously is harmless to guinea pig.	+ (10 min)					0	0				0	0
Collargol	Colloidal silver			do	+ (4 min)					0	0				0	0
Argentamin	Silver phosphate diethylendiamin.	6±	Not precipitated by albumin.	1 part to 1,000 of body weight given subcutaneously is harmless to guinea pig.	+ (immediately)	0	0			0	0				0	0
Protargol	Silver and albumose	8	Precipitated by albumin. Albumin not coagulated.		+ (1–3 min)	+ (1–3 min.)	±			0	0				0	0
Argonin	Silver and casein	4	Not precipitated by albumin.	1 part to 10,000 of body weight given subcutaneously is harmless to guinea pig.	+ (1–3 min)		+ (20 min)			0	0				0	0

Nargol	Silver and nucleic acid.	10	Not precipitated by albumin. Albumin not coagulated.	1 part to 1,000 of body weight given subcutaneously causes local necrosis only in guinea pig.	+ (1 min)	+ (1–2 min.)	+ (3 min.)	+ (2–4 min.)	±	0		0						0	0
Albargin	Silver and gelatose	15	Affects albumin slowly	1 part to 10,000 of body weight given subcutaneously is harmless to guinea pig.	+ (1 min)					+ (1 min)		0						0	0
Ichthargan	Silver-thio-sulphonate.	30	Precipitated by albumin, but precipitate is redissolved in excess of albumin, Albumin not coagulated.	Less toxic to man than silver nitrate (Aufrecht and Wood). 1 part to 5,000 of body weight subcutaneously is harmless to guinea pigs.	+					+ (1 min)		+ (8 min.)	0					0	0
Actol	Silver lactate	50	Decomposed by proteids, but said to form soluble compounds. Albumin not coagulated.	1 gram can be given to man subcutaneously without serious symptom (Wood). 1 part to 10,000 of body weight given subcutaneously is harmless to guinea pig.	+					+ (immediately)		+ (1 min)	±					0	0
Silver nitrate	Silver nitrate	63+	Precipitated by albumin. Albumin coagulated.	Average therapeutic dose for man is 0.01 gram (U.S.P.).	+					+ (immediately)		+ (2–15 min)	±					0	0

SUMMARY AND CONCLUSIONS

1. These tests have determined quantitatively within narrow limits the balantidicidal action of the substances tested.

2. It has been demonstrated that the compounds of arsenic and antimony, the analine dyes, ipecac and its alkaloid emetine, and quinine, substances which are employed more or less successfully in the treatment of other protozoan diseases, possess little or no balantidicidal value.

3. The salts of the heavy metals, especially mercury and silver, have been found to be eminently balantidicidal.

4. It is possible that some of the inorganic salts of mercury or silver, administered by mouth, or by subcutaneous or intraveneous injection, might be efficient in the treatment of balantidiasis. The salts of mercury are successfully employed in the treatment of certain spirochæte infections, and when given internally are eliminated in part by the mucosa of the large intestine; consequently, the mercury would be brought in direct contact with the infected tissues in balantidiasis.

5. The application of these inorganic salts of mercury and silver to the local treatment of balantidiasis is rendered impracticable by the facts that they are precipitated by albumin, and consequently possess little power of penetrating the tissues, and that they are relatively toxic for man.

6. The organic compounds of silver are not precipitated by albumin or, if precipitated, form soluble compounds that should be capable of penetrating the tissues, and they are relatively nontoxic for man.

7. Quantitative tests have demonstrated that certain of these organic compounds of silver possess a balantidicidal activity as great, in proportion to the amount of silver contained, as silver nitrate.

8. The practical value of these organic compounds of silver in the treatment of balantidiasis can be determined only through clinical experience.

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THE RELATIONSHIP OF VARIOLA AND VACCINIA

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(From the United States Army Board for the Study of Tropical Diseases as they Exist in the Philippine Islands)

From Jenner's day until the present the relationship of variola and vaccinia has been a subject of frequent, often violent, and always unsatisfactory discussion, and no explanation of the relationship has yet received general acceptance. We think that an explanation that is simple, complete, and satisfactory is here presented. The prolonged absence of smallpox from Manila has hitherto prevented our undertaking certain experiments that we think might afford definite proof of our view. Therefore, we advance the view, supported by facts already known, and hope that others may be able to do the experimental work, although we shall undertake it in case we can obtain smallpox cases.

I. BASIC FACTS

The following basic facts as to the smallpox-vaccinia relationship merit first mention.

1. Smallpox contagion or inoculation gives rise in man to smallpox, a highly contagious, generalized disease of considerable mortality, characterized ordinarily by a preëruptive stage, and other stages related to the appearance, development, and subsidence of the eruption.

2. Passed through monkeys and cattle for a few generations and brought back to man, the virus gives rise to vaccinia, a localized, noncontagious, mild disease, that in itself causes no mortality, although septic complications may cause some.

3. Having, by passage, once lost its power to produce smallpox, the virus *never regains* it, even though passed from person to person (proper hosts for variola virus) for thirty-five (1) or one hundred (2) (3) years.

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II. EXPLANATION OF BASIC FACTS

We can think of two possible, complete, and satisfactory explanations for the above facts.

1. The germ of smallpox by passage through certain lower animals loses (acquires) certain properties, and it transmits its altered condition to its offspring forever, a more striking instance of hereditary transmission of acquired characteristics than has ever before (so far as we know) been cited.

2. *Smallpox is due to a dual and divisible virus, one part of which causes vaccinia and the specific smallpox eruption, the other part being necessary for the production of the contagious, generalized, mortal disease with a distinct preëruptive stage and initial rashes.*

We are unable to imagine a third explanation [2] that does not ignore facts now known; of the two set forth above we favor the second for two reasons:

A. Without desiring to enter into the discussion of the hereditary transmission of acquired characteristics and without professing to have made a thorough study of the subject, we

[2] Kelsch and his coworkers(5) apparently hold the view that variola virus and vaccinia virus are distinct entities, but are derived from a common stem. This view ignores, and Kelsch appears to deny, the possibility of transformation of smallpox to vaccinia by animal passages, a fact that we consider clearly established.

Meirelles(6) advances the view that smallpox is flea-borne and argues at length in favor of his belief. Under this hypothesis it would be understandable that the smallpox virus undergoes a sexual stage of development in the flea and that vaccinia is milder because it lacks that stage. But we think this disproved by several facts; namely:

1. Inoculated smallpox never changes in man to vaccinia.(5)

2. Variola may be transmitted to man after 3 or even more animal passages that have all been made by inoculation.

3. Vaccinia has never been known to start a smallpox epidemic, even in Meirelles' own flea-ridden country.

4. Strains of vaccine have been kept pure as such, by human passages, since Jenner's day, probably too long a period for an asexual phase of a normally sexual organism to live.

Supfle(7) says that the vaccine germ remains localized and only leads to a local manifestation and reproduction. The immunity is purely histogenic and is limited to the epithelial layers that are physiologically closely related to the inoculation site. The nature of the attenuation of variola to vaccine lies in the fact that the growth intensity of the germ is lowered by calf passage. The immune bodies are developed and appear soon enough to prevent generalization of the locally inserted vaccine germs. This hypothesis ignores the fact that vaccinia *never regains* the power to produce smallpox, even though it become generalized at times.

incline to the opinion that nontransmission of acquired characters by heredity is a general law.

B. We think that already known facts, to be set forth in this paper, point rather to the correctness of the dual virus hypothesis.

III. EVIDENCE SUPPORTING THE HYPOTHESIS

1. MORE OF LESS ANALOGOUS CLOSE ASSOCIATION OF VIRUSES

Hog cholera was long ascribed to *B. suipestifer*, the disease was stated to be produced by its inoculation, and extensive vaccination was practiced as a preventive measure.(4)

Yet now it is considered to be due to a filterable virus, and the bacillus merely occurs in close association with that virus. Possibly other diseases (distemper of dogs, guinea-pig epizoötic of Petrie and O'Brien, and scarlet fever) present a typical disease picture only as a result of the combined action of filterable virus and bacteria.(8) Prowazek and Baurepaire(9) think that smallpox virus acts in symbiosis with streptococcus.

The original street virus of hydrophobia, uniformly fatal to man, may be changed by numerous passages through rabbits to *virus fixe*, which causes a much shorter incubation and earlier death in rabbits, yet this fixed virus may be used to immunize man. Högye's method of using fresh virus diluted has had extensive trial;(10) and it is even stated(11) that the entire brains of rabbits dying from inoculations with *virus fixe* may be and have been injected into men without harm resulting, and that the use of considerable doses of fresh *virus fixe* has given excellent results in 92 cases in the Allegheny General Hospital.(12)

This indicates a close analogy between smallpox and hydrophobia, and suggests to us that hydrophobia may be due to a dual and divisible virus.

2. ANIMAL POXES. VARIOLATION OF ANIMALS

There are a number of animal poxes, the most important being smallpox, cow-pox, horse-pox (grease), sheep-pox, and swine-pox.

We know, from trial and personal observation, that man, ox, horse, sheep, and swine, the respective natural hosts of these diseases, are all subject to vaccinia.

Immermann (3) says that man is occasionally subject to ovination, the result being a single pock, and says further:

> We have already mentioned and considered in detail the fact that cattle are readily inoculated, not only with humanized vaccinia and human

variola, but also with sheep-pox and horse-pox. The reaction to all these procedures is, without exception, the production of a local pustular eruption, which, when inoculated back into the original animal, likewise causes nothing but a local affection capable of further cultivation throughout the species. Finally, the temporary or permanent protection conferred upon individuals of any species (human or animal) by successful inoculation with any of these varieties of virus extends to all forms of smallpox, whether they be animal or human.

It would appear from this that the pock-causing element is common to these diseases and that they are mutually protective one against the other; yet that smallpox and sheep-pox, both highly contagious and highly mortal diseases in their respective natural hosts, are not the same is indicated by the fact that man does not get sheep-pox and sheep do not get smallpox. It is further indicated by the pathologic findings in sheep-pox.(13) (14)

While touching the relationship of animal poxes we may here mention, on the authority of Doctor Ruediger, in charge of the serum laboratory, Bureau of Science, Manila, that the strain of vaccine virus now used by that Bureau and by the Government of the Philippine Islands, a strain with which almost 7,000,000 vaccinations have been made with great success, was derived from a fatal case of human smallpox in 1908, having been passed first through monkeys, and from the second monkey to a heifer, and from the fifth heifer to man.

Gauducheau(15) reports a similar experience from French Indo-China.

For many years the common origin of smallpox and vaccinia was disputed, largely because numerous able investigators were unable to inoculate cattle satisfactorily, or at all, with variolous lymph.

Kelsch(5) reports 20 failures in 1909 and 1910.

Copeman(16) gives a good account of the early work.

At present it is conceded that direct inoculation from variolous man to cattle is often, if not usually, unsuccessful, and the more satisfactory method of obtaining variola-vaccine is first to inoculate from man to monkey and later from monkey to cattle, although Freyer(17) reports success from the use of rabbits instead of monkeys. From this it might be presumed that the bovine host is normally less susceptible and more resistant to smallpox virus than is the monkey, and the same is probably true of vaccine virus, as in our experience, with 16 monkeys and 9 cattle, the vaccine pustules are uniformly much larger in the monkey, as they also are in man.

The heifer being more resistant to vaccine virus, it follows that virus growing on the heifer must be able to withstand the greater resistance there offered, only the more vigorous and counter-resistant virus will survive, and by that process of selection the virulence of the strain becomes exalted, a conclusion supported by the following observations:

Brinckerhoff and Tyzzer(18) found that vaccinia more thoroughly protected monkeys from subsequent vaccination than did a previous attack of variola inoculata, although both protected against later variola inoculata. They quote Roger and Wiel as having made similar observations.

Dupont(19) found 22 per cent of 2,601 Sudanese, who had had variola vera or variola inoculata, still susceptible to vaccinia, although he says that the immunity of these people to smallpox is usually complete and permanent.

Schamberg(20) quotes Martin as having obtained 35 per cent of successful vaccinations with old, long humanized virus, and 80 per cent with animal virus and early human removes.

It is stated(2) that strains of vaccine passed from man to man, in China, for a hundred years, are almost inert. However, Immermann says that bovine lymph also deteriorates when passed exclusively from bovine to bovine host. Still, that does not affect the fact that the pock-forming element of smallpox virus first becomes exalted by passage through cattle.

Under our hypothesis there is another element in that virus, apparently one without which it cannot produce a contagious, generalized, mortal disease with a distinct preëruptive phase and initial rashes. This element dies or is otherwise eliminated by the animal passages, but such elimination does not always occur on the first passage.

Copeman and Immermann both discuss this matter rather fully, and agree that smallpox occasionally may be transmitted from cattle to man after two or three passages.

Brinckerhoff and Magrath(21) state that they carried a strain of variola virus from man to monkey, thence through 4 generations on rabbits' corneas, and then back to a monkey, causing smallpox, with the formation of a propustule and a secondary eruption. We know of no instance of smallpox production at a later remove than this from the human case. A single bovine passage may suffice to change the virus to vaccine.

It may be inferred, we think, that although the whole disease, smallpox, does not occur in cattle, the whole virus may live in them for a time, the separation of elements not always occurring suddenly.

This gradual separation of elements of a virus may be cited as an instance of selection, rather than one of either hereditary transmission or double virus. To this we reply that a virus offering so wide a field for selection is, in our view, dual and divisible.

We may here mention that a recent report by Simpson(22) of the apparent simultaneous occurrence of rinderpest and smallpox in an Indian buffalo, taken in connection with the case of Private Vann, to be mentioned later, caused us to think that possibly rinderpest might so weaken the general resistance of cattle as to allow vaccinia to become generalized on them.

Thanks to the coöperation of the Bureau of Agriculture of these Islands, we have been able to vaccinate 9 Batan or Luzon cattle, either before, at the time of, or shortly after their inoculation with rinderpest. We have obtained localized vaccinia and rinderpest, but no generalization of vaccinia. With a piece of scab the size of a grain of wheat, taken from the vaccination site of one of these animals, triturated in water, and inoculated into numerous small skin incisions, we have produced good, localized vaccinia and fatal rinderpest in another, but no generalization of vaccinia.

We hope to experiment soon with a buffalo.

3. CLINICAL OBSERVATIONS ON SMALLPOX

In 1899 Private Vann of the Twenty-third Infantry was admitted to the military hospital at Cebu, P. I., in a very low state from sprue. Death appeared imminent, and although the man was awaiting transportation to the United States it was feared that he would not live long enough to reach there. Another soldier, suffering from fever, vomiting, headache, and backache was admitted to the ward, and placed in the bed adjoining Vann's. He died of purpura variolosa in a few days. The condition having been suspected to be variola the day following the admission, all men in the hospital were at once vaccinated. Within a time now thought to have been not more than a week (the notes made in 1899 are not available), Vann showed a generalized eruption resembling discrete smallpox. Concomitantly he expressed himself as feeling better and asked for solid food which he ate without discomfort or injury. The improvement begun then continued, and a complete recovery from the sprue followed rapidly. The man was still in the service late in 1911 as a sergeant of the Hospital Corps.

This case was interpreted at the time and since as one of generalized vaccinia, the generalization occurring because of

the patient's low resistance, a consequence of the extreme state of debility resulting from the sprue.

The evidential value of recollections of thirteen years ago is recognized as slight, but the facts are essentially as stated, and the case is reported as being of interest, at least.

There are three irregular forms of smallpox that are characterized by a total lack of pock formation or by a very brief and atypical pock stage. They are (1) purpura variolosa, always fatal, (2) varioloid or modified smallpox, and (3) *variola sine eruptione*.

Variolous purpura is described by practically all writers on smallpox, and there is general agreement that it proves fatal before any eruption appears. It is not to be confounded with that form of hæmorrhagic smallpox in which the hæmorrhages occur into the pocks (hæmorrhagic pustular smallpox). The investigation of a large number of writings does not show unanimity of opinion as to the protective value of vaccination against this form of the disease, but, as the form itself is rare and as a few positive observations outweigh many negative ones, we think it safe to say that vaccination does not protect against it.

Bancroft (23) had 12 cases among 1,200 of smallpox. Of these, 3 were unvaccinated; 7 had been vaccinated in childhood, of whom 3 had good foveated scars; and in 2 vaccination had been attempted, without success, two weeks prior to the onset of the disease.

Armstrong (24) says:

> A history of previous vaccination, unless recent, does not play apparently a very important rôle in this variety of the disease, as the infection is of such severity that all resistance to immunity is overcome.

He reports one case in the person of a discharged soldier, who, because a soldier, had presumably been successfully vaccinated, though the fact is not mentioned.

MacCombie (25) says:

> I have not met with a case in any one who had one-third of a square inch of well foveated vaccination cicatrix, and who had been successfully revaccinated.

Osler (26) reports 27 cases, of whom 13 had been vaccinated, but none revaccinated.

Kaposi (27) says:

> Vaccination does not appear to offer the slightest protection against this form of the disease.

Meirelles(6) reports 2 cases in persons successfully vaccinated within five years. Without desiring to discuss at present this writer's hypothesis that smallpox is flea-borne, we quote the following statements as valuable because based on very extensive clinical observation:

> The evolution of the blood phase of variola is similar in vaccinated and unvaccinated.
>
> The eruptive phase is benign, almost absent, or rapid and slightly pustulous in the vaccinated, even when confluence of macules and papules promises confluent pustulation; it is grave, on the contrary, in the greater part of the nonvaccinated.

He then reports a case that we may include here as one of severe varioloid, or possibly hæmorrhagic smallpox modified by vaccination.

> A German, aged 45 years, vaccinated in his own country on his entrance to school and revaccinated later on entering military service, was admitted during my service.
>
> He had fever of 40°C. with intense headache and backache, pains in all the body, vomiting, delirium, etc., like other variola cases. The third or fourth day all his body was covered with macules and papules of smallpox, so confluent that there was not a patch of sound skin the size of a pinhead. The diagnosis of confluent smallpox was necessary. * * * Notwithstanding the enormous confluence of macules and papules that enabled one to foresee confluent and abundant pustulation, a half dozen only, on the face and chest, became pustules of the size of a pinhead, at the center of the papule; all the others disappeared; their red color darkened progressively to black, while the macules diminished in size, so that toward the end of the disease the German had his body covered with black points.
>
> One sees that the hematic phase of smallpox in this patient, vaccinated and revaccinated, was developed with the same intensity, with the same symptoms as in the nonvaccinated; the eruptive phase, above all the pustular, which in the nonvaccinated is usually grave and abundantly purulent, was nothing, or insignificant in him. * * * I could cite still other cases of variola similar to this, all vaccinated, where the hematic phase was intense and where the confluence of macules and papules indicated a grave infection, that nevertheless terminated with no or insignificant pustulation. I do not recollect seeing a single similar case in a nonvaccinated individual.

Vaughan(28) asks:

> Why is it that in a protected case suffering from an affection that is practically nearly universal, and almost confluent everywhere on the trunk, one not infrequently finds practically no secondary fever, whereas a case with a similar rash in an unprotected subject would give an abundant secondary fever and would prove not by any means a matter for congratulation, nor would it offer grounds for a prognosis such as may amply be justified in a case protected by vaccination?

Concerning mild smallpox (*varioloid*) Councilman says:

The initial period may be typical and severe or mild. The symptoms suddenly abate and are followed by an eruption that may consist of only a few pocks. Welch and Schamberg report a case in which but a single pock appeared. The pocks are usually small and superficial and may be readily overlooked or their nature unsuspected. * * * Cases of confluent and purpuric smallpox are just as apt to follow infection by these mild cases as from any other form. * * * Such cases are now rarely found except in vaccinated individuals.

Immermann says:

Fever, delirium, and other combinations of initial symptoms are often as violent as could be wished. * * * The differences between mild and grave variola (varioloid and variola) become more decided with the eruption of the smallpox exanthem, that is, in general from the end of the third day of the disease—and from that on the differences are found at least as much in the general as in the local symptoms.

The condition of the body temperature and the general condition are pathognomonic for decided cases of varioloid. Immediately with the first appearance of the smallpox exanthem on the skin the fever begins to abate, and the fall of the temperature is generally so rapid and so complete that on the fourth day of the disease complete apyrexia has already made its appearance.

Bancroft(23) says:

In some instances an initial fever of the most severe type was present, accompanied by delirium and unconsciousness, and continuing for four or five days.

Variola sine eruptione is recognized by practically all writers. Councilman(29) says:

It appears as an illness of an indefinite character, occurring chiefly in hospital attendants on the twelfth day after exposure to smallpox. The symptoms consist in headache, pain in the back, fever and nausea. They may be so slight that the individual pursues his ordinary vocations, or they may approach in severity an ordinary initial stage. The symptoms last two or three days and then suddenly abate. The condition was well marked in one of the physicians investigating the Boston epidemic in 1901. Characteristic initial rashes may appear during the attack. One patient, a pregnant woman, remembered having a headache about two weeks after exposure to the disease, but was not otherwise affected. Her child showed a typical eruption when two days old. A group of three cases which appeared in one of the large hospitals in Boston, the onset in whom was nearly simultaneous, was traced to a ward tender who had an attack of what was supposed to be grip.

There were 12 of these cases among Bancroft's 1,200. Osler(26) says:

They seem to have been not uncommon in recent epidemics.

MacCombie(25) says:

> I have only seen such cases in vaccinated, sometimes in revaccinated, subjects.

Wilson(30) says:

> Variola sine eruptione occurs in young persons who have been well vaccinated.

We think that the above quoted clinical observations indicate that *vaccination protects against the eruptive, and especially against the pustular stage of smallpox, rather than, or to a greater degree than, against the whole disease, smallpox.* This, if true, would afford strong support for our hypothesis.

The statement does not in any way imply that the value of prophylactic vaccination is less than has been thought, but does explain some, if not most, of the apparent failures, and also explains the successes resulting from its use; for, with the exception of variolous purpura, smallpox principally kills by, in, or as a result of, its pock stage.

We do not at this time desire to discuss the conclusions of Councilman, Calkins,(31) Prowazek, Casagrandi,(32) (33) (34) and others as to the cause of smallpox and the nature of the germ. It may be noted, however, that Calkins and Councilman cause *Cytoryctes variolæ* to transmit acquired (loss of) characters, in that by bovine passage it forever loses the power it once possessed of entering the nuclei of ectodermal cells and of undergoing a sexual cycle.

Prowazek's "initial bodies" (Chlamydozoa)(35) are endowed by him with the power to transmit acquired characters.

Whether or not the work of any one of these or of any other investigator of the cause of smallpox be correct, it may be so only in so far as it relates to the common element in vaccinia and variola.

That there is such a common element is shown by the mutual protection afforded by inoculation of the two viruses, by the common histologic and other microscopic findings, by the mutual deviation of complement,(36)(37) and probably by allergic reactions.(38)

Thus far we know of no work and no observations that necessarily controvert our hypothesis. Should this hypothesis prove to be a truth, it might be found applicable to many diseases, and it would certainly provide a viewpoint from which they should, at least, be considered.

POSTSCRIPT

Only since writing the above article have we been able to obtain Ricketts and Bayles' Diagnosis of Smallpox. These authors, while not entertaining our views as to the dual and divisible nature of smallpox virus, and holding strongly that vaccinia protects against the whole of smallpox, nevertheless do consider and speak of smallpox as a "dual disease," and say, "The fever proper of smallpox is that of the septicæmia, and the local rash and the secondary fever bear the same relation to it as the pneumonia to measles, or the adenitis to scarlet fever." Inasmuch as the pneumonia of measles and the adenitis of scarlet fever are probably always due to invasion of bacteria distinct from the viruses of the specific diseases, we regard the analogy as fairly good. It would be better could the pneumonia of measles or the adenitis of scarlet fever be shown to be due always to one specific virus (as is the pock of variola), and could that virus, by cultivation or by growth in animals, be obtained in a relatively pure and harmless condition and used to immunize healthy infants against the entire disease, or the more serious part of the disease, measles, or scarlet fever.

Using Ricketts and Bayles' nomenclature in explaining our view, we may say that *smallpox* is due to virus *AB*. Of these (or the parts of this), *A* is mainly responsible for the "toxic fever" and "toxic rashes," although possibly the combination *AB* is necessary before *A* can manifest itself; *B* is responsible for the "focal eruption" and for vaccinia. *A*'s action is first manifested, *B*'s follows, and it may be followed in turn by a third cause of injury, for instance, a streptococcus or staphylococcus infection. Complete immunity to *B* should constitute at least partial and possibly complete immunity to *AB*, although, should *A* be capable of acting alone, immunity to *B* would not affect it.

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THE BIONOMICS OF STOMOXYS CALCITRANS LINNÆUS; A PRELIMINARY ACCOUNT

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INTRODUCTION

I have had unusual opportunity to study the bionomics of *Stomoxys calcitrans* in connection with a large number of experiments on the transmission of surra, where thousands of flies of this species were captured or bred. It has been my aim not to duplicate the information presented by Newstead[2] in his excellent treatise on this subject, and the present paper may be considered as supplementary to it. I have attempted to present the subject in as nontechnical a manner as is feasible.

OVIPOSITION

Age at which the fly lays eggs.—This was definitely determined in two instances in flies bred in the laboratory. In both the time was the same. The females were found with 2 males in copulation, April 11, when they were removed from the

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[2] *Ann. Trop. Med. & Parasit.* (1907), 1, 82–96; reprinted from *Journ. Econom. Biol.* ( ), 1, 158–166.

breeding jar and kept in individual test tubes. Two days later, April 13, eggs were found in both tubes. These flies had emerged April 4 and had been fed daily on a monkey. Their actual age at the time of egg laying was 9 days. Another fly which emerged from its puparium on March 21 laid its batch of eggs, 81 in all, on March 30, when 9 days old.

Number of eggs laid by a single Stomoxys.—Under normal conditions the eggs of *Stomoxys calcitrans* are laid in the manure of its host. The eggs have been found in the fæces of the horse, the carabao, the bullock, and no doubt are to be looked for in the fæces of all domesticated animals. Under laboratory conditions guinea-pig manure offers the best medium for egg laying and subsequent development. In the laboratory, under artificial conditions, this insect deposits its eggs in ordinary glass tubes and on filter paper under glass. These ova hatch in due time if moisture be furnished them.

In order to determine accurately the number of eggs laid by a single female, not much reliance could be placed on observations under field conditions; therefore, it was necessary to resort to experimental procedure. At first captive gravid flies were used for this investigation; then an attempt was made to verify the findings with flies bred and mated in the laboratory.

The flies collected in the open were usually taken from healthy work animals and placed at once in test tubes plugged with cotton. By using numbers of females of various ages, it was hoped to obtain approximately the number in one which had not yet made an initial oviposition. From these eggs flies were reared for the purpose of checking the total oviposition in the wild flies.

Under these artificial conditions the females laid eggs in the glass tubes beginning as early as the second day of their captivity. The greatest number of eggs laid at one deposition was 94, while 5 flies laid respectively 82, 86, 91, 91, and 94 eggs. In every instance the eggs laid proved fertile, and the larvæ from them were transferred at once to breeding jars with suitable food.

The flies used for this study were kept in the dark at a uniform temperature not exceeding 22° C. They were transferred daily to clean vessels and fed on monkeys and guinea pigs. Seven of the number which survived beyond ten days furnish the following data: Egg deposition extended over a period which, in 3 instances, comprised the entire life of the flies during captivity; in 2 others, to within three and four days of the death of the flies. Two flies escaped after having laid, respectively, 446 and 438 eggs.

In two cases recorded, as many as 20 batches of eggs were laid by a single fly, and the greatest number of eggs deposited by an individual was 632. In this instance oviposition occurred one day preceding the fly's death, which took place on the sixty-fifth day of its laboratory history.

TABLE I.—*Data on oviposition.*

Length of time fly was kept.	Number of depositions made.	Total number of eggs.
Days.		
50	7	168
34	9	182
60	20	438
72	13	435
65	20	632
70	11	318
64	15	446

In addition to the eggs laid, there were present after death a substantial number in each of the females dissected. In three of these the contents of the egg chambers in the dissected ovarian tubes were counted. In order to simplify the count, the chains of immature ova were not taken into consideration. It was found that the contents of the ovaries of the fly which had laid 632 eggs consisted of 90 ripe eggs and 98 partially developed eggs, making a total of 820 for 1 female. This number, 820, may be fairly accepted, I think, as the maximum number of eggs produced by a female *Stomoxys*. Twelve bred flies were employed for enumeration in oviposition. In these cases the eggs produced were sterile. As the result of these counts, no information can be added to that obtained previously. The total number of eggs deposited by any laboratory-bred female did not approach the maximum of the depositions made by the captive flies.

If the contents of the ovarian tubes can be accepted as a criterion of the possible maximum of egg production, one instance among the new flies would appear to establish the record in this regard. A fly emerging April 1 laid 106 eggs previous to its death, which occurred May 14. The dissected contents of the ovaries showed 71 ripe eggs, 112 half-developed eggs, and 840 immature eggs in various stages of development; a total of 1,123 from 1 female. However, until further investigation, this number will not be accepted as the possible maximum number of eggs that would be deposited by a *Stomoxys*. I prefer to consider 820 as the more authentic number.

The egg is creamy white and of the ordinary muscid type, with its convex side adhering to the place of attachment when laid. The grooved concave side through which the larva makes its exit is conveniently considered the dorsal side. The length of the egg averages 1 millimeter. There is no appreciable change either in color or form during incubation, which under ordinary conditions takes from twenty to twenty-six hours.

THE PROCESS OF HATCHING

In observing the hatching microscopically, it is quite necessary for the best results to suspend the eggs in a moist medium, preferably, physiological salt solution. When fertile eggs are placed in the air on a dry slide for as long as one hour, the hatching which has begun is inhibited and the embryo dies within the egg.

A certain sign of sterility, which can be applied as a test for eggs of this species, is the absence of embryonic movement within two days. There is not a distinctive dark polar spot, and the color changes to a dull pink, then rose, followed by brown after two days. If some moisture is present, the sterile egg ordinarily does not shrivel.

The movements of the embryo usually cannot be observed until the last four hours of incubation, when the cephalic area becomes ash-gray and gradually darkens as the movements become more active. The usual dilatation and contraction of the chorion take place accompanied by the occasional movement of the amniotic fluid.

Two pharyngeal spines, which appear under the cuticle, curved slightly posteriorly on the pharyngeal apophyses assist the embryo to escape from the egg. Prior to the process of hatching, the embryo lies curled up, and as the body gradually straightens out the head capsule extends from its sinus bringing the hatching spines into contact with both slits of the micropyle canal, the cephalic end of which is neatly carved out, laying open a flap of the chorion of the egg. By means of pressure from the posterior end and a constant prodding of the head appendages, the micropyle canal is forced open and the larva glides through the opening.

The ova are very sensitive to changes of temperature, light, and humidity. Incubation can be lengthened to fully double its normal length by lowering the temperature or by withdrawing the moisture from the medium in which eggs are laid. Exposure to light also influences the metamorphosis of this fly. At a room temperature of 30° to 31° C., eggs hatch in

from twenty to twenty-six hours. At a temperature of 20° to 22° C., eggs hatch in from forty-eight to sixty hours, depending on the humidity. Eggs kept in a darkened closet hatch four to six hours sooner than individuals of the same brood kept at the same temperature in a room exposed to light from windows.

The influence of moisture on the incubation period may be illustrated by the following observation. A fly was placed in a test tube while in the act of laying eggs. Forty eggs were deposited, distributed along the sides of the tube. The first egg was observed to be laid at 11.40 a. m., and the remainder during thirty minutes. A piece of filter paper half the length of the tube was soaked with salt solution and placed with the eggs. Note was taken where the first egg of the batch was laid, which was about 3 centimeters from the end of the moistened paper and close to the end of the cotton plug. At 1 p. m. of the following day the eggs at the bottom of the tube, where the moisture accumulated, commenced hatching. All of the eggs, except the first one laid, hatched before 2.30 p. m. of the same day. This egg hatched upon the day following at 11.20 a. m., nearly one day later than the eggs in the moistened end of the tube. The larva from this egg appeared quite normal; it was observed to crawl immediately toward the moisture at the bottom of the tube. This phenomenon appears to be in keeping with notes made of field conditions, and when flies are reared in glass vessels in the laboratory.

LARVAL LIFE

The young larva loses no time in consuming whatever desirable food may be present. The feeding seems to be continuous, broken only by the short period of seclusion just before the formation of the pupa. Food seeking is relatively a passive process with this insect; the mother provides for the life of its progeny, in laying the eggs only where there is an abundant food supply.

Very little time is consumed by the larva in adapting itself to food conditions. The bloody excreta of the mother, if present, is first consumed, then the more inviting portion of the animal manure is devoured. The color of the insect, at first waxy or creamy white, rapidly assumes the color of the ingested food. This is pale green at first, changing, from the posterior end to the anterior portion, to pale brown. The larval cuticle does not become darker before six to seven days.

The larvæ thrive on many kinds of food. The following materials were found experimentally to produce healthy adult flies: Manure from the horse, cow, carabao, and guinea pig; guinea grass; bran; bran and horse manure; corn-meal and horse manure; and horse manure saturated with blood from the horse and monkey.

It was found that ordinary filter paper served as food for the omnivorous larvæ. The paper was invariably placed over the manure in the jar to assist in regulating the moisture content, and it was noticed that within three days the paper was full of holes and jagged along the edges. In about five days the filter paper was represented by a few scattered strands which disappeared usually before the larvæ matured. It was demonstrated that larvæ could develop on filter paper soaked in manure decoction, provided the latter was supplied fresh daily.

The development of molds in the breeding jars interferes with the growth of the larvæ, but the appearance of some fungi does not usually produce any effect. Indeed, the spore heads of certain fungi, which are commonly a part of the flora of this manure, seem to be greatly relished by larvæ of *Stomoxys*.

It was found to be advantageous to boil all of the ingredients with the exception of the blood in the culture jars, in order to destroy the animal and vegetable life, especially the mites found commonly in manure, and various species of mold, inimical to the fly's development.

CANNIBALISM

The parasitic tendencies of this fly are developed at an early age. The first manifestation is shown in the young maggots which when confined to a test tube will invariably remove the moisture from each other's bodies. When the food in the breeding jar is allowed to become dry, the larvæ clump together and lick the moisture from each other's bodies. This is only a step toward the stage where portions of the body are removed and a state of cannibalism results.

Two instances were observed of larvæ feeding upon other injured larvæ. On one occasion 2 nearly full-grown larvæ were seen feeding on the juices exuding from a large jagged hole torn in the side of an injured, but still active maggot. It was not ascertained if the 2 feeders were responsible for the injury, but the injured larva was soon helpless and became an easy prey to its fellows.

DEVELOPMENT OF THE LARVA

Stomoxys calcitrans remains in the larval stage under ordinary laboratory conditions for a period averaging twelve days. Between the third and fourth days the larva makes its greatest growth in length. By the sixth day the larva has reached its maximum thickness.

TABLE II.—*Development of a typical larval Stomoxys.*

Date.	Period of development.	Length.	Width.
		mm.	*mm.*
April 9	After hatching	1.0	
April 12	Third day	3.5	
April 13	Fourth day	6.0	0.75
April 15	Sixth day	7.0	1.5
April 17	Eighth day	9.0	1.5
April 21	Twelfth day	10.0	1.5
	Full grown		

THE FORMATION OF THE PUPARIUM

The puparium is formed two days after the larva has attained its maximum size, which under ideal conditions is from the eighth to the twelfth day. The puparium is constructed without any apparent sloughing or shedding of the larval skin, the process being one of simple contraction. A larva measuring 10 millimeters is reformed slightly, and contracts to 5 millimeters. The body is thickened from 1.5 millimeters to 2 millimeters. There is an invagination of the cephalic end bearing the head capsule, and this and the anal end become broadly rounded.

The full-grown larva is coated with a glossy, slimy cuticle which is pale chrome yellow in color. It lies inert at the beginning of the somnus which lasts until the puparium is formed. The viscera wrinkle and disintegrate and assume the yellowish color of the Malpighian tubules and the cæcal glands. Soon the color of the cuticle blends with that of the internal structures, becoming pale clay yellow. The only structures now visible are the lines of the trachea and the dark brown anal stigmatæ. During the changes indicated the mouth cavity is constantly kept in slight action accompanied by a barely perceptible general telescopic movement. Upon the following day these activities cease, the barrel-like puparium is completed, and no internal organs are visible. With the absence of movement, the buccal cavity has become invaginated and is covered by the cap of the

puparium. This end of the insect is red-tipped, while the remainder is decided golden in color. The color of the whole puparium changes rapidly to burnt sienna.

The encapsuled puparium is usually from 5 to 9 millimeters in length, depending on the nourishment and care the larva has received. The female has a larger puparium than the male fly, and the female is as a rule 0.5 millimeter longer than the male from the same lot.

The male fly takes less time than the female to pass the nymph stage. In more than 40 instances recorded, first emergences were marked by the appearance of males. The male fly usually precedes the female by two days.

INFLUENCES OF ENVIRONMENT ON THE DEVELOPMENT OF THE NYMPH

Certain artificial conditions have been found to affect the developing nymph. When kept in water during the whole of this stage, 30 pupæ failed to develop; nor did they develop when removed from the water after seven days and placed in a dry glass after drying on filter paper. The lowering of the temperature from that of the room at an average of 29° C. to that of the cold room at 21° C. retarded emergence two to four days, but seemed to increase the percentage of emergences. An exposure to the outdoor light and sunshine at a maximum temperature of 43° C. killed the developing nymphs, while the light of the room at the same temperature of the darkened cabinet inhibited the development to the extent of two to three days.

It can be inferred from these experiences that the optimum conditions for pupal development include a dark, cool, moist medium; and these conditions prevail where the puparium occurs in nature.

The length of time spent in the puparium is fairly constant. It is usually never greater than five and one-half days under natural conditions, but when modified by the artificial environment of the laboratory this period may be extended to six or even to ten or twelve days.

EMERGENCE OF THE FLY

The pupa gives no indication of movement of any sort, such as occurs in some orthoraphous flies, which might be interpreted as premonitory of emergence. The only sign of activity, to inform the observer of what is occurring within, precedes emergence only by a minute or two, and consists of a barely perceptible rising and falling of the operculum or cap. Immediately

there follows a splitting of the cleavage lines at the cephalic end of the pupal envelope. A slit appears in the fourth segment encircling the puparium, isolating an apical section of a cone which is also divided by a median line. Usually one-half of this raised cap serves as a lid which, opening, allows the fly to escape.

The subsequent development prior to flight is divided into 3 stages which are so graphically described by Newstead [3] that I refrain from repetition, and refer the reader to his paper in which this phase of the life history as well as many others are treated with his usual faithfulness and clearness of description.

In emerging, sometimes the imago is held at the anal segment by a tissue which at first sight appears to be the lining of the puparium. This is the exuvia of "the final ecdysis" (Newstead) which the fly attempts to part with when it leaves the puparium. In some cases the emergence is effected with the effete skin intact, when it is plastered to the anal end of the fly and remains attached even after the insect takes its initial flight.

It has been observed in a few cases that if at this stage, prior to the unfolding and hardening of the wings, the fly be immersed in water for two minutes or more, development ceases.

The sexes are readily distinguished upon emergence. The female is invariably the larger and the lighter in color. It emerges with its long tapering ovipositor projected until the body drys thoroughly and flight is begun.

To show the time required before a fly is able to take care of itself after emerging, the following chronological note is appended:

Morning of April 30, 1912.

9.35: The operculum has been split and the fly, a male, released from the puparium.

9.40: Length, 5 millimeters.

9.42: The wings unroll and separate from the body, where they are held while drying and hardening. Since emerging, the proboscis is held against the notum between the 2 interlocking processes of the mesotrochanters.

9.52: The labellum of the proboscis is seen to change from the ultra ash gray to brown, then to black.

9.55: The entire labium becomes brown.

9.58: The proboscis has been detached from the thoracic clasp through movements of the legs. When released it gradually drops and swings in place through traction by the longitudinal muscles. This organ then assumes the normal position under, and anterior to, the head.

[3] *Loc cit.*

10.00: The length of the fly, including the projection of the labium, has increased to 5.5 millimeters.
10.03: The fly makes its initial flight inside of the flask.
10.10: The proboscis is now jet black and fairly hard.

From these notes it can be seen that it requires one-half hour of drying before the insect is able to fly and also that it would be impossible for the fly to apply its proboscis in feeding for an equally long time after emergence.

The difference in the time of emergence of flies from the same lot of eggs is usually out of all proportion to the difference of time of deposition of the eggs. The larvæ from a lot of eggs laid by a collection of females on June 24 to 26 commenced pupating July 2 and emerged July 15. Emergence continued daily up to July 26, fully eleven days after the first appearance of flies. It was noticed that the flies appearing last were fully 1 millimeter smaller than those appearing first. This was due perhaps to the gradual drying of the food medium which was less suitable for nourishing the larvæ hatching last.

This difference in size is seen also in flies of precisely the same age. This was noted in 2 females emerging April 4, fed daily upon the same animal. When 20 days old they measured, respectively, 5.5 millimeters by 2.5 millimeters, and 7 millimeters by 3 millimeters.

FEEDING HABITS OF STOMOXYS CALCITRANS

In my experience the adult fly, male and female, can be kept alive only by feeding on the blood of animals. Drawn blood, although accepted, apparently does not answer the requirements of the fly, even when renewed daily. Flies nourished in this manner usually do not survive longer than flies kept without food.

Under laboratory conditions flies of this species will feed for the first time six to eight hours after leaving the puparium, but I do not doubt that, in nature, blood is taken as early as one hour after emergence. Several laboratory-bred flies have been seen feeding in an apparently half-hearted fashion for a few minutes within one hour after emerging.

Judging from observations made under experimental conditions, *Stomoxys* is essentially a blood feeder; it has never been observed to take plant juices, although it sips water when confined in jars and test tubes.

Under conditions obtaining in these Islands, the fly will feed readily on man, although it rarely attacks him in the presence of

domesticated animals. It is rarely found to annoy man to the extent that prevails in temperate climates. Its attacks upon man generally take place shortly after the atmosphere has been cooled by a rain shower and at certain seasons of the year when this species is unusually abundant. The following notes are added to show the extent of the attacks of these flies on man when they are abundant.

At 8.50 a. m. August 27, 1912, a female *Stomoxys calcitrans* flew into the laboratory after a rain shower, alighted on my exposed arm, and in a few seconds commenced to scrape the skin with its labellum. Within ten seconds sharp pain was felt. The probing continued for two minutes when apparently a satisfactory insertion of the proboscis was effected. At this time the distention of the abdomen of the fly became apparent. The aspirating process caused only a dull pain. Although the blood gushed into the stomach of the parasite the labium was inserted to only one-third its length. The base of the labium was not inserted nor was the labium buried in the skin to the bulbous portion as is usual when this fly is feeding on other mammals.

The fly under observation fed for three minutes and thirty seconds. A blood drop the size of a pinhead was left at the site of feeding, and one hour later a very slight pain was felt, while a minute hæmorrhagic spot marked the place of feeding. The bitten area on the arm was marked with a blue paraffin pencil, and within an hour another fly settled within the boundaries of the blue mark. It fed one minute and a few seconds, during which time a third fly appeared and made a bite only 4 millimeters distant from the blue mark, and fed for two minutes.

While I was jotting down these notes, another fly, the fourth parasite, visited the bitten arm and selected a spot within the marked area bitten by its fellows. All four of the foregoing parasites were males. Less than ten minutes elapsed when a fifth *Stomoxys* appeared on the bared arm, and commenced operations within a centimeter of the area bitten by the last fly. In this instance the parasite was a female, and, from appearances, one which had been subjected to a long fast. This fly required nearly two minutes to aspirate any blood. In six minutes the labium was inserted to the maximum depth, that is, to the bulb, and the fleshy portion of the labium leading into the

pharynx kept up a constant titilation. At no time was the labium held still, but there was a continual piston-like movement. The fly bit for twelve minutes and forty seconds, defecating at intervals of from thirty to forty seconds, and at each deposition the fluid voided was bloody. The first evacuation took place synchronously with the first dilatation of the abdomen.

The termination of the biting was followed by a quick withdrawal of the labium to its middle, then the rest was slowly withdrawn and cleaned on the fore tarsi. A blood drop flowed to the surface of the skin at the site of feeding. No pain was felt when the labium was withdrawn. All of the bites occurred in an area of 3 centimeters on the fleshy part of the left forearm. I do not attempt to explain why these parasites showed such a marked predilection for a restricted area of my arm. The same phenomenon occurred again subsequently.

Stomoxys calcitrans appears to attack all animals with equal avidity. Sick animals especially are marked for their attacks. It has been observed by many investigators that infected animals, particularly horses, are more susceptible to the attacks of *Stomoxys* than are healthy horses. During an outbreak of surra, two years ago, it was noticed in a public corral that 3 horses among a large number of work animals contracted surra, probably several days before the malady was diagnosed. The attention of the veterinarian in charge was attracted, primarily, not by the clinical symptoms, but by the large number of biting flies present on one of the sick animals. This horse did not attempt to dislodge the parasites, the flies feeding until engorged, then flying to the nearest fence to rest. Three horses were examined for blood parasites, and many trypanosomes were found. The weakest horse showed the greatest number, and concomitantly the predominance of the ectoparasites. Incidentally, when the 3 sick horses were removed, the infection among the remainder of the animals was checked.

Experimentally *Stomoxys* will feed on any animal offered for this purpose. Twenty-four specimens of *Stomoxys calcitrans* were taken while observed in the act of biting a horse. They were placed in 24 glass tubes and applied each day, as long as they survived, to a different species of animal. In every instance the flies bit and fed upon the blood of the animal on which they were placed. The following table shows the number of flies which survived after each meal and the average number of minutes each fly fed on the various hosts.

TABLE III.—*Showing the feeding of Stomoxys on various animals.*

Date.	Animal used.	Number of flies fed.	Length of time fed (average per fly).
			Min.
October 16	Horse	24	3.5
October 17	Monkey	23	4.0
October 18	Carabao	20	3.0
October 19	Bullock	18	4.0
October 20	Goat	15	2.5
October 21	Sheep	14	3.5
October 22	Guinea pig	11	3.5
October 23	Pig	11	2.5
October 24	Cat	11	4.5
October 25	Deer	10	2.0
October 26	Dog	10	3.0
October 27	Rabbit	10	2.5
October 28	Chicken	10	2.5
October 29	Bat	8	2.5
October 30	Rat	7	4.5
October 31	Lizard (gecko)	4	8.5
November 1	Man (Filipino)	4	3.0

The table shows that at least 4 of the flies fed on 17 species of animals in as many days.

The only fact of practical value suggested by these experiments is that *Stomoxys calcitrans* will accept any host which will submit to its attacks. Therefore, a sick animal would be most exposed to the bites of *Stomoxys* or other insects of similar habits. The inference to be drawn relative to epidemiology is obvious.

THE FEEDING RELATION OF NONBITING FLIES TO STOMOXYS

A peculiar feeding relation has been observed to exist between *Stomoxys* and certain nonbiting flies. I was curious to learn why such large numbers of nonbiting flies were generally found in collecting insects from domesticated animals. Moreover, in an examination of extensive collections made with a net swung over the backs of the animals, the majority of the nonbiting flies were found to have blood-engorged abdomens. When these were dissected and examined microscopically, mammalian blood was found to be the principal food constituent.

A quiet bullock was selected for closer observation. On this animal some 150 to 200 flies, mostly muscids, were seen. Many hundred dung flies, including house flies, were scattered about on the floor of the stall, and occasionally one of these joined the blood-sucking flies on the body of the bullock.

perature of 20° C. to 23° C. No special provision was made for moisture in the tubes. Under these conditions the maximum period of life was seventy-two days. This was a female.

In the tests with laboratory-bred flies, which emerged about the middle of July, 63 flies were employed. Twenty-four males had an average longevity of twenty-eight days, while 39 female flies lived for an average of thirty-two days. Six of the females showed an average longevity of fifty-four days, and 6 of the males averaged thirty-nine days. One female of this series lived seventy-two days, while 1 male lived fully ninety-four days.

Perhaps a fairer method to determine the natural longevity would be to mark thousands of bred flies and set them free, and from time to time, through systematic collecting, attempt to recover them.

MATING

The mating of *Stomoxys calcitrans* was never observed under field conditions except in one instance where a pair was observed in copulation while the female was attempting to feed. The process was observed in bred flies in 3 instances, occurring in 2 pairs upon the seventh day after emerging from the puparium. As has been noted previously, two days after the copulatory act, fertile eggs were laid. These flies had been kept in company with many others in a large bottle which was daily applied to a monkey for feeding purposes.

In the act of mating the male assumes the active rôle, flying off after a period of ten minutes. In these flies the male is above, almost at right angles, adhering only by the genitalia and front legs, the other legs being suspended at each side. The clasping organ of the male exerts a pressure upward and forward, the female genitalia respond in a backward and downward movement, resulting in a quick, decided telescoping of the parts. The male withdraws the intromittent organ by a downward pull at the conclusion of the process. It releases its hind legs and flies away directly while the female remains for a few minutes. During this time a reflex telescopic action takes place until the invaginated parts, which have been depressed, are extended to their normal length.

METHODS EMPLOYED IN KEEPING AND FEEDING FLIES FOR LABORATORY PURPOSES

The methods employed for keeping and feeding *Stomoxys* in captivity will serve for other species of blood-sucking flies; for example, species of *Lyperosia* and of the Hippoboscidæ. The

greatest difficulty has been encountered in attempting to keep flies in a common enclosure.

Screened stable.—In a screened stable, aside from the artificial conditions of confinement, the difficulties are chiefly from the presence of natural enemies, and do what one may it is well nigh impossible to wholly eradicate them. Particular reference is made to the common insectivorous lizard and the ubiquitous spider. Spraying with pure creoline was partially, but not wholly effective.

Glass vessels.—Large bottles and museum jars of a minimum capacity of 3 liters were used when it was desired to confine and to feed at one time a considerable number of flies. Thirty days was the longest time that flies were kept in these containters. In this instance, it was found necessary, for the preservation of life during the last ten days, to transfer the flies to individual test tubes after feeding.

In the use of large glass vessels, untimely death resulted from mite infestation, cannibalism, and excess of moisture.

An unknown mite, not restricted to these flies, was found to be parasitic, both in the hypopial stage and in the adult form, upon the flies. The first of these stages did not prove a menace unless present in great numbers either on the body, which precluded proper functioning of the spiracles, or on the proboscis, which prevented the insertion of the labium in feeding. When the mite was present as a true parasite, in the adult form, an occasional one or two did not seriously affect the fly, but when present in large numbers they were sufficient to enfeeble it.

Cannibalism was encountered in the experiments to an unusual degree. Often the disability of an individual fly attracted the attention of another more active member which promptly attempted, and usually succeeded, in puncturing the helpless fly's abdomen. This disability might result from engorgement, infirmities resulting from broken labium, or from the wings adhering to the glass due to an excess of moisture. I have found numerous cases of flies actually fracturing the labium in attempting to penetrate the host's epidermis, and it may result from the fly pricking at the glass in attempting to sip moisture from the container. Such a condition, of course, makes feeding impossible as the proboscis is not rigid enough to puncture the skin, and as a result the fly dies from starvation.

Where a large number of flies are quartered it is difficult to avoid an excess of moisture even though a bibilous filter paper is employed. The condition is probably the result of excretory

contamination and the condensation of the moisture in the air in the bottle, when kept at a temperature of from 20° to 28° C. The excess of moisture causes the flies to become stuck by their wings to the sides of the bottle where they soon die.

The use of individual glass tubes.—This method has proved the most successful for keeping *Stomoxys* in captivity. The fly can be observed at all times, and its longevity is increased to nearly the normal. Ninety-four days was found to be the maximum life of adult flies kept individually in glass tubes. A test tube of 24-millimeter bore, plugged with cotton, was found the most convenient sort. A piece of white filter paper conforming to the size of the tube was found ideal to regulate the moisture, and this was changed at least every two to three days. It was found advantageous to change the fly to a fresh tube not oftener than twice each week. In feeding it was not found necessary to screen the mouth of the tube. The filter paper was first removed, the base of the tube was directed toward the window light and the tube was inverted immediately over the animal's body. The fly after feeding was induced to release its hold upon the skin of its host by gently tapping the tube and gradually inclining the latter toward the light, after which the filter paper was restored and the tube stopped with a cotton plug.

The flies when not fed were kept in the dark at a temperature between 20° and 28° C.

METHOD OF APPLYNG THE FLIES TO THE HOST IN FEEDING

Monkeys.—The following method was pursued in applying large numbers of flies in a bottle. The monkey was strapped, abdomen down, to an improvised stock by means of surgical gauze or twine. The wrists and ankles, which were bandaged previously to prevent chafing, were first secured; then the tail was closely cropped, bound to a stout wire with straps of gauze, and thrust into a narrow-necked bottle which contained the flies to be fed. The other end of the wire was kept at a convenient distance from the mouth of the bottle to facilitate manipulation. Wiring the tail was necessary to prevent the animal from switching that appendage against the glass and crushing numerous flies.

In feeding the flies from test tubes, the tubes were inverted over the thigh or other convenient part of the monkey. At least 2 flies could be fed at once in this manner.

Guinea pigs.—When this animal was subjected to fly bites in a large museum jar it was found to be of advantage to im-

mobilize it by strapping to a frame of brass wire. This was done in order that movements of the animal would not interfere with the biting of the flies or with the observation of the flies throughout feeding. Cropping the hair of this host was found to facilitate the feeding of the flies. It was necessary to hold the museum jar horizontally with the bottom toward the light. Here the majority of flies assemble when not feeding, and the light reactions of the fly are taken advantage of in withdrawing and introducing the host. If desired, ether can be used to advantage in the transfer of animals. It should be applied at the screened end of the jar, lightly enough to prevent flight, but not sufficiently to stupefy the insects. The flies can also be fed individually in test tubes to guinea pigs strapped to stocks, being applied to some convenient part of the body, preferably the side of the abdomen.

Horses.—The method more commonly employed by investigators is to enclose both the flies and the horse in a screened stall or shed. Here it is not possible to make close and accurate observations, and despite the fact that many thousands of flies could be kept at once they did not, in my experience, live longer than eight days, and usually died in five days even when food was constantly available.

By strapping the horse to an operating table, accurate data of the feeding process can be obtained. This method supplanted the crude one of throwing the horse to the ground and feeding flies from inverted bottles. The violent struggling of the horse under these conditions is not conducive to making accurate observations.

In all of the methods of feeding the flies, the hair of the host was closely cropped with scissors. It was found advantageous, also, to slightly dampen the skin of the host to make the animal odor more attractive to the insect and to arouse its blood-drawing instincts.

SUMMARY

1. The age at which *Stomoxys calcitrans* begins egg laying has been determined in bred flies to be nine days.

2. The maximum number of eggs produced by a single *Stomoxys* may be stated as at least 632 and possibly 820. As many as 20 depositions may be made in the lifetime of a female.

3. The incubation period for these eggs is from twenty to twenty-six hours at a temperature of from 30° to 31° C.

4. The larval stage under optimum conditions is usually from seven to eight days.

5. The imago emerges from the puparium generally in five days.

6. The fly of either sex takes its initial bite in from six to eight hours after emergence. Flies of this species have fed experimentally on 17 species of vertebrates including mammals, reptiles, and birds.

7. In feeding on live stock, *Stomoxys calcitrans* makes a wound with its labium from which nonbiting flies suck blood.

8. The female may live at least seventy-two days and the male ninety-four days.

9. The development of *Stomoxys calcitrans*, as shown by Table IV, varies considerably, depending upon the environment. Under optimum conditions, it is twelve days.

TABLE IV.—*Life history of Stomoxys calcitrans at various periods under various conditions.*

Date of oviposition.	Incubation period.	Larval stage.	Pupal-stage.	Life cycle.	Conditions of development.
	Days.	*Days.*	*Days.*	*Days.*	
February 7	2	26	6	35	Medium of dry horse manure left in light of the room.
February 17	2	14	7	23	Do.
February 23	2	11	6	19	Do.
Do	1	13	5	19	Do.
April 7	2	14	6	22	Do.[a]
June 14	1	8	5	14	Medium of moist horse manure and corn meal.[a b]
August 10	1	9	5.5	15.5	Medium of moist horse manure and brand.[a]
October 1	1	7	5	13	Medium of moist guinea-pig manure mixed with chopped guinea grass.[a]
October 12	1	9	5	15	Medium of moist horse manure and layers of filter paper.[b]
October 23	1	6	5	12	Medium of carabao and horse manure placed in a barrel shaded at all hours.[a]

[a] These 5 cultures were developed in open jars in an airy closet darkened at all hours.
[b] From this brood several flies emerged one month after egg laying.

GENERAL CONDITIONS AFFECTING THE PUBLIC HEALTH AND DISEASES PREVALENT IN THE BATANES ISLANDS, P. I.[1]

By David G. Willets

(*From the Biological Laboratory, Bureau of Science, Manila, P. I.*)

The following report is based upon observations made in the Batanes Islands, chiefly in the town of Santo Domingo de Basco, Batan Island, from April 2 to May 7 inclusive, 1912. Much of my information was received from the padres, of whom there are seven in the province, and from the provincial *cirujano administrante.*[2]

The Batanes are located about 240 kilometers north of the Island of Luzon in the typhoon belt, and scarcely a year passes without considerable damage being done to homes and live stock for this reason. Several very strong currents about the islands render communication between the various members of the group not infrequently extremely dangerous and at times impossible. Lives are lost almost yearly in these currents.

The population is approximately 8,000, distributed as follows: Batan Island, 5,200; Sabtang Island, 1,300; and Itbayat Island, 1,500. It is said that at one time the total was about 20,000, but that emigration to various parts of Luzon is responsible for the great reduction.

The occupation of the inhabitants is cattle raising; a few devote their time to fishing. Cattle raising is conducted on a small scale, about 1,000 head being sent to the Manila market annually. The average price paid the natives for their cattle is about 25 pesos per head. I am informed that the cattle are paid for frequently with cloth, rope, rice, etc., which are sold

[1] Read before the Manila Medical Society at its June, 1912, meeting.

[2] A *cirujano administrante* is a person who has studied medicine in the Santo Tomás University of the city of Manila for a period of not less than two years. He may be registered to practice medicine in remote towns of a province where no civilian doctor of medicine or licenciate of medicine is available. Upon passing a satisfactory examination before the district health officer for the province in which he desires to practice, he pays to its provincial treasurer a fee of 10 pesos for a certificate of registration which shall thereupon be issued to him by the district health officer.

at a shameful percentage above the retail Manila price. The islands, particularly Itbayat, have plenty of pasture land, and cattle raising could be carried on somewhat extensively. Land suitable for garden purposes, however, is scarce and widely distributed over the islands, hence the population must always be a limited one.

The inhabitants have been spoken of as hard working and industrious. My observations do not support this statement. It is true that they must of necessity put in long hours of labor, but this is largely due to the fact that the cultivable land is widely scattered. A great deal of time is, therefore, required to go to and from the fields or rather little garden patches. Having secured something to eat and some clothing, the people seem to be quite content. Day laborers are difficult to secure at any reasonable wage, and the workmen are slow. It is customary for several members of a family to go to the fields in the early morning and return about sunset or later. Some of the families go to the fields on Monday morning returning Saturday afternoon. During the week it is not unusual for them to rest during the day and work at night if there be a moon. Little shacks are put up in the pastures for their protection during these periods.

The homes on Itbayat are made of coral rock foundation, plank floor, and sides and roof of cogon grass. On Batan and Sabtang they are made of coral rock with cogon grass roofs. Rarely a shack is found on these two islands. The better home consists of two parts, a kitchen and a dwelling portion, but in the vast majority of cases there is only one room to serve as kitchen, dwelling-room, and bedroom. Inside of this room a wood fire is made on a few stones and, since the house is unprovided with a chimney, the smoke at times becomes almost unendurable and the dirt is frightful. The great majority of the homes are poorly ventilated, and it is the custom of the natives, as elsewhere in the Philippines, to close their homes as tightly as possible at night. Sudden changes in the temperature are not rare, and there is not sufficient fuel available to keep a continuous fire when it is indicated. Hence the houses are uncomfortably cool and damp at times. The small amount of money at its disposal and the exorbitant prices prohibit a family from having enough clothing for the cooler portions of the year.

Cattle, hogs, goats, and chickens are almost the only possessions of this people and naturally are guarded carefully, especially during the night, when they are kept close to the house. Indeed it is not a rare event to see the family pig or the family

goat inside of the house. There are only twenty-one horses in the province; one on Itbayat and twenty on Batan. Dogs are very numerous. At Sabtang the cattle are kept in corrals at night, because the houses are situated too closely together to permit of their being kept in the dooryard.

A suitable method for the disposal of human excrement is lacking. An effort is now being made to provide water-closets, but as yet they are insufficient for the number of families and furthermore the people have not learned to use them; this applies particularly to Santo Domingo de Basco and Mahatao, Batan Island. On Sabtang and Itbayat Islands, water-closets are very rare.

Each of the islands of the group is poorly supplied with fresh water. Of the six towns only one, namely Mahatao, can boast of a stream. Raile, a barrio of Itbayat, also has a small stream. The other streams of the islands are too far removed from the towns to provide drinking water; these are, however, of importance in furnishing water for cattle. Several springs are to be found on the various islands, but these also are too remote from the towns to furnish drinking water excepting one near the town of Sabtang. Usually rain water is used for drinking purposes. This is collected in earthen jars from the roofs of houses and from trees by means of pieces of split bamboo. The water for miscellaneous purposes is obtained from a limited number of wells and cisterns.

The water jars and cisterns are good places for mosquitoes to breed. On Itbayat there are marshy places which also doubtless serve this purpose. Flies were not very numerous.

Santo Domingo de Basco, Mahatao, and Ibana on Batan Island, and Sabtang on Sabtang Island are each provided with two street cleaners.

From the facts that fresh water is scanty, that the one room serves usually as kitchen, living-room, and bedroom, that domestic animals are kept near the house, and that the natives, excepting the inhabitants of Sabtang, do not care to bathe in sea water, it is clear that these people are not especially noteworthy for their cleanliness.

The diet consists of camotes and fish caught in the Batanes waters and dried; fresh fish is seldom eaten. In addition, a few miscellaneous vegetables are used and meat of one kind or another—beef, pork, goat-meat, or chicken—is eaten on an average of about twice a week. Some eggs are also used. On Itbayat, a rather limited supply of oranges, bananas, pineapples, and coconuts are available in season. Practically the only

pleasure of the people is in drinking *palek*, a native alcoholic beverage made from sugar cane, and in smoking tobacco which is raised for their own consumption.

Basing my opinion upon a rather scanty knowledge of the natives in various parts of the Philippines, I believe the general physical condition of the inhabitants is above the average. This is attributed to their occupation which keeps them in the open a great deal of the time. The women of Itbayat appear to be particularly strong and vigorous. Statistics show that the death rate is about 26 per thousand per annum and that the infant mortality is about 30 per cent. Illegitimate children are very common when one considers that the communities are very small, ranging from about 200 to 2,000. In some of the towns such births make up 30 per cent of the total birth rate. None of these statistics are considered to be reliable. Adult females are more numerous than adult males, the ratio being about 5 to 2 in Santo Domingo de Basco; this is explained by the greater tendency of males to emigrate.

A great deal of the morbidity is connected with the respiratory tract. Due to sudden changes of temperature, dampness in the homes during the rainy season, exposure while fishing or working in the fields by moonlight, and insufficient clothing for the cooler portions of the year, one cold after another is contracted from childhood up so that bronchitis, which is not infrequently associated with spitting of blood but without afternoon temperature, night sweats, or notable loss of weight, is common. This condition, coupled with the fact that homes are habitually closed during the night and that many persons sleep in the same room, creates a favorable soil for tuberculosis. As a matter of fact, "phthisis" is understood by the adult population to be common. A number of cases of this disease were seen. From answers to questions asked, I believe pneumonia claims its victims each year and that pleurisy is not rare.

Careful search for cases of paragonimiasis failed to disclose a single case. Dr. A. G. Sison of the Philippine General Hospital tells me he knows of a case of this disease occurring in a native of the Batanes.

Skin diseases are very common, especially chronic ulcer. Several persons were seen who were incapacitated for work because of the extent to which the ulceration had progressed. Probably not less than 50 persons were seen during my short sojourn in the islands who were suffering from this affection. The duration of the disease in these cases varied from a few

months to twenty years. Ringworm is common. Two cases of herpes zoster were found. Three patients were suffering from poisoning similar to poison ivy, but in each case the process seemed to be more severe than that caused by poison ivy. The marked frequency and severity of skin disease is attributed largely to the uncleanliness of the inhabitants.

Rheumatism is a rather common disease. Several cases of the chronic form were seen, and the previous history of a number of persons presenting themselves at the clinic suggested the occurrence of this disease.

Bright's disease is also a common affection.

Upon first meeting residents of the Batanes the writer was told of a rapidly fatal fever endemic and at times epidemic on the Island of Itbayat. The disease is said to be more frequent from July to December than in other parts of the year, and it is the consensus of opinion of those best informed relative to its occurrence that it has been less common during recent years than formerly. It is claimed, however, that deaths occur every year from the disease and that the residents of Batan and Sabtang who visit Itbayat are especially apt to contract it. Several persons from these two islands have undoubtedly died of the fever shortly after returning from Itbayat, but the number of such deaths has been greatly exaggerated. Probably not exceeding an average of one person a year from Batan and Sabtang has died from this cause during the past ten years.

Investigation revealed the facts that the disease is characterized not only by fever but also by chills, vomiting, and sweating. Hence malaria was suspected. However, in the examination of a number of persons from Itbayat only one enlarged spleen was found. The blood of this patient, as well as that of a number of others from Itbayat and a few from Batan who claimed to have had the fever within the past two years, was examined for malarial organisms with negative results. The only case in which malarial parasites were found in the blood was in an acute infection contracted by the writer, apparently on Itbayat Island. The parasites were of the æstivo-autumnal type, and the objective symptoms present were recognized by several residents of Santo Domingo de Basco as being identical with those exhibited in typical cases of the Itbayat fever seen previously by them. I became convinced finally that the "fever" so greatly feared by the natives is probably malarial fever of a pernicious form. Further investigation is considered necessary to prove this definitely.

The presence of the malady on the Island of Itbayat has an important bearing on the development of the province. Itbayat is far more fertile than any of the other islands of this group, but the inhabitants of Batan and Sabtang will not move to Itbayat for fear of the fever. Of course, the natives think they have *the* fever every time they have a temperature from any cause whatsoever. It is thought that benign tertian malaria is present. The term *paludismo*, malaria, is certainly well understood by the natives, and quinine is a drug with which many families are familiar.

No case was seen suggesting elephantiasis, and the examination of the blood taken at night from 191 adults for the presence of microfilaria resulted negatively.

Despite the fact that dysentery is not infrequently given by the provincial *cirujano administrante* as the cause of death of both adults and children, a distinct history of dysentery was very rare. No acute case was seen and only 2 chronic ones; each of these was negative for entamœbæ. Occasional diarrhœa is common. The stools of 400 inhabitants of Santo Domingo de Basco composed of 100 adults each, and 100 children each, of both sexes, were studied statistically for evidences of intestinal parasitism. Two thin cover-slip preparations were examined of each case. A carthartic was not administered prior to taking the specimens.

The specimens were unfavorable for an examination for the presence of protozoan parasites. Only 5 such infections were found; namely, 1 with monads, 2 with entamœbæ, and 2 with *Balantidium coli*. These findings coupled with the rarity of a history of acute or chronic dysentery argue for the infrequency of entamœbic infections. Two infections with *Balantidium coli* in such a small number of examinations is unusual and far in excess of the average in other statistical studies in the Philippines. These cases were found in adult males, each of whom had visited Manila for a short period.

Evidence of helminthic infection was readily found in each and every one of the 400 individuals examined. Single infections were present in 46 per cent; double in 42.5 per cent; triple in 11.5 per cent; *Ascaris* in 92.8 per cent; *Trichuris* in 46.7 per cent; hookworms in 24.5 per cent; *Oxyuris* in 1 per cent; and *Strongyloides* in 0.5 per cent. No cestode infections occurred in the 400 persons examined, but one infection with *Tænia saginata* was found at Sabtang.

In over 19,000 persons examined in various parts of Luzon by sundry investigators for evidence of intestinal helminthiasis

an average of 85.66 per cent was found to be infected.[3] The highest percentage (95.9) was found by Garrison, Leynes, and Llamas[4] in the study of 1,000 inhabitants of Taytay, Rizal Province. In these studies the average *Ascaris, Trichuris,* and hookworm infections were 61.36, 40.79, and 30.57 respectively. It is, therefore, clear that the high rate of infection with intestinal worms in the Batanes is due to the great frequency of ascariasis. The results of the present study are given in Tables I and II.

TABLE I.—*Frequency of infection with intestinal worms in the 400 persons examined.*

Sex and age.	Examined.	Infected.	Infections.		
			Single.	Double.	Triple.
Children:					
Male	100	100	46	44	10
Female	100	100	51	43	6
Adults:					
Male	100	100	45	42	13
Female	100	100	42	41	17
Totals	400	400	184	170	46
Percentage		100	46.0	42.5	11.5

TABLE II.—*Frequency of infection with various parasites in the 400 persons examined.*

Sex and age.	Examined.	*Ascaris.*	*Trichuris.*	Hookworm.	*Oxyuris.*	*Strongyloides.*
Children:						
Male	100	93	50	18	2	1
Female	100	96	44	14	0	1
Adults:						
Male	100	87	42	38	1	0
Female	100	95	51	28	1	0
Totals	400	371	187	98	4	2
Percentage		92.8	46.7	24.5	1.0	0.5

A part of an epidemic of measles was observed. The infection had been carried to the Batanes by a child who had been in Manila for medical attention in January, 1912. There were numerous cases of measles in Manila at that time. The epidemic was confined to the town of Santo Domingo de Basco.

[3] Willets, *This Journal, Sec. B* (1911), 6, 77.
[4] *Ibid.* (1909), 4, 207.

Thirty-five cases had been reported up to the time I left the islands. Probably many more cases had occurred.

Chicken-pox was present several years ago. No histories were secured suggestive of typhoid fever, diphtheria, or scarlet fever. Cholera was apparently present in 1902. Dengue fever occurs from time to time according to the testimony of the *cirujano administrante*. Beriberi is apparently absent. No cases suggestive of this disease were seen, and no suspicious histories were obtained. The last case of smallpox seems to have been in 1896. About 2,000 persons were vaccinated while I was in the islands, and sufficient virus to vaccinate 2,000 more was left with the *cirujano administrante* on May 7. This quantity was sufficient to bring the vaccination up to date. Three cases of insanity were seen, and the history of 4 others secured. No case of yaws was seen.

Since 1906 several lepers have been taken from the Batanes by the Bureau of Health. Some of these were native to the islands, while others were fugitives from northern Luzon. No case of leprosy was found during my investigation.

One apparently typical case of migraine was found. The patient stated that a cousin and an aunt were similarly affected.

One case strongly suggested the occurrence of cerebro spinal meningitis in the islands about four years ago. The patient was a boy 5 years old who had been quite normal until one year of age, when he became acutely ill. His temperature was very high, tremors and convulsions were common; and his grandmother recognized the opisthotonus condition at once and said the child had such a symptom. She also stated that about 10 other children had the same disease about the same time and that they all died. The head, body, and upper extremities of the patient were well developed. The lower extremities were considerably undersized. The patient had never been able to walk, and he was mentally very backward, being able to talk only with great difficulty and his vocabulary was limited.

Venereal disease is rare. Not one case of undoubted clinical syphilis was seen. Some of the old ulcerous cases may have been syphilitic, but a Wassermann reaction would have been necessary to render a positive diagnosis. Four cases of gonorrhœa were found, 2 of which were in Filipino cooks who had recently arrived from Manila.

Death claims yearly its victims among the new born and mothers because of the lack of medical attention during par-

turition and the latter part of pregnancy. I have no reason, however, to believe the death rate from this cause to be greater in the Batanes than elsewhere in the Philippines where physicians are unavailable.

Foreign growths were found in several cases. Three cases of cataract, 1 of probable gastric ulcer, and one of probable chronic appendicitis were also seen. From 15 to 20 cases were found which would be benefited by the surgeon's knife.

A STUDY OF THE NORMAL BLOOD OF THE CARABAO [1]

By WILLIAM HUTCHINS BOYNTON

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The following study was undertaken with the object of ascertaining the normal condition of the circulating blood of the carabao, so that one desiring to make a clinical examination of the blood might have a standard for comparison.

In searching the literature on the subject at my disposal, I have not been able to find any previous work done on the blood of carabao, which circumstance necessitated a systematic study of a large number of healthy animals.

The twenty-five animals used were in apparently normal condition. Their temperatures were taken twice a day, several weeks before the examinations were made. The animals averaged from two and one-half to 6 years of age. Some were work animals used at the laboratory, and had been immunized to rinderpest from six months to two years previous to the time of examination. The majority were susceptible to rinderpest and were kept at the laboratory for experimental purposes.

The blood was obtained in all cases from the ear. The part was first thoroughly cleaned with water, then dried with alcohol, and one of the small veins on the outer side of the lobe was pricked with a sharp-pointed scalpel.

The red corpuscles were counted by means of Thoma's hæmatocytometer, using Toisson's diluting fluid. The corpuscles in 100 squares in each of 2 slides were counted. If these counts did not agree closely, a third preparation was made, and the results of the three were averaged. The leucocytes were counted in the same preparation as the red cells, the counting chamber used having the Zappert-Ewing ruling. The percentage of hæmoglobin was obtained by means of the Tallquist hæmoglobin scale, as no other apparatus was available. The specific gravity was obtained by Hammerschlag's method. The time of coagulation was obtained by Wright's method. The relative volume of corpuscles and of plasma was obtained by the hæmatocrit as modified by Daland. This was placed in a centrifuge, and revolved at a speed of approximately 3,000 revolutions per minute for three minutes. Films were made on glass slides, and fixed

[1] Reprinted from Bulletin 21, Bureau of Agriculture of the Government of the Philippine Islands.

[2] Archibald R. Ward, chief.

with heat, in pure methyl alcohol or in equal parts of absolute alcohol and ether. The slides were stained with eosin and methylene blue, Ehrlich's triacid mixture, Jenner's stain, and Wright's stain. Jenner's stain was used in making the differential counts, as it is easily handled, and gives very accurate results. The size of the corpuscles was obtained with a Zeiss ocular screw micrometer, using stained films, and taking those parts of the film in which the cells were not crowded.

The technique used in staining the preparation was as follows:

When Jenner's stain was used, the film was dried in air and flooded with the dye, which was left to act for from two to three minutes. It was then washed from ten to fifteen seconds in distilled water, and dried as rapidly as possible in air. When well stained and washed, the red cells had a terra-cotta color.

With Wright's stain, the film was dried in air, and flooded with the dye which was allowed to act for one minute. Distilled water was then added, drop by drop, until a film of metallic luster began to form on the surface. This was allowed to act from two to three minutes longer. The preparation was then washed for ten seconds in distilled water, and dried in air as rapidly as possible. With this stain, the red corpuscles took a pinkish color.

With eosin and methylene blue, the film was previously fixed, either with heat or by submerging in pure methyl alcohol for ten minutes, or in equal parts of absolute alcohol and ether for ten minutes. Then it was flooded with saturated alcoholic solution of Ehrlich's blood eosin for about ten seconds and washed in water. After this it was flooded with a saturated aqueous solution of Grubler's methylene blue for one minute, washed quickly with distilled water, and dried in the air.

With Ehrlich's triacid mixture, the film was previously fixed by placing the slides, film side down, upon a heated copper tray, and held at a temperature just below boiling for fifteen minutes. The film was then covered with the mixture and allowed to act ten minutes, after which it was washed hastily in distilled water and dried rapidly in air.

The red corpuscles in the fresh condition appear as biconcave disks of a homogeneous appearance, yellowish in color, and with a nearly translucent central area and have the general appearance of human blood. With eosin and methylene blue and with Wright's stain, the red corpuscles take a pinkish stain, with Jenner's they are terra-cotta color, and with Ehrlich's triacid mixture they have an orange tint.

Five varieties of leucocytes were noticed in the circulating

blood, which correspond very closely to those found in cattle. Following the widely used classification they are (1) lymphocyte, (2) large mononuclear, (3) polynuclear, (4) eosinophile, and (5) mast cell.

Lymphocytes include cells averaging 7.3 microns in diameter, being a little larger than the red corpuscles, and having a nucleus occupying a greater part of the cell body. The nucleus is usually round, but may show a notch on one side. The cell body shows as a narrow rim around the nucleus. Both nucleus and cell body are coarsely reticular. The cell body has a strong affinity for basic stains, and the nodal points in the reticulum take a deeper stain. With Jenner's and Wright's stains, a few small purplish granules are frequently seen in the cell body. With eosin and methylene blue, both nucleus and cell body take a deep blue stain, often the cell body staining deeper than the nucleus. With Ehrlich's triacid mixture, the nucleus takes a greenish color, the cell body has a purplish tinge, and appears homogeneous. This variety of cell is nongranular, except for the few purplish granules numbering from 1 to 5 frequently seen, as has been previously mentioned.

Large mononuclear leucocytes include cells which are considerably larger than the lymphocytes, averaging 10.8 microns in diameter. The nucleus occupies from one-half to two-thirds of the cell, and is situated at one side of the center. It is either oval or kidney-shaped. Both nucleus and cell body are finely reticular, and stain less deeply than do those of the lymphocytes. Frequently small clear areas or vacuoles are seen in the nucleus, giving it the appearance of undergoing degeneration. With Ehrlich's triacid mixture, the nucleus takes a greenish tinge, and the cell body has a very faint pinkish tint. With eosin and methylene blue, the nucleus is light blue and the cell body is distinctly blue, but neither nucleus nor cell body takes such a deep stain as the lymphocytes. With Jenner's stain, both nucleus and cell body are stained blue, but not so strongly as in the case of the lymphocytes. With Wright's stain, the nucleus is dark violet, and the cell body pale blue.

Transitional forms of the large mononuclears were noticed frequently. In these cells the nucleus is saddlebag-shaped. The staining properties are similar to those already described for the large mononuclears.

In polynuclear leucocytes the nucleus takes on various shapes. It may be S-shaped, W-shaped, Z-shaped, coiled or lobulated, and is coarsely reticular. In appearance they are very similar to those in human blood. The cell body remains practically un-

stained, but contains many fine granules, which are so small that they appear as mere points, and show a rather weak affinity for acid stains. The granules are more numerous than the coarser granules in the eosinophiles. These cells average 9.4 microns in diameter, or nearly twice the diameter of the red corpuscles.

With Jenner's stain, the nucleus is blue and the granules are a bright pink. With Wright's stain, the nucleus has a dark violet color and the granules are distinctly pink. With Ehlirch's triacid mixture, the nucleus takes a pale greenish color, and the granules a pinkish violet. With eosin and methylene blue the appearance is similar to that described for Jenner's stain.

In eosinophile leucocytes the nucleus is very similar to that of the polynuclears, usually being bilobed or trilobed. As a rule the nucleus takes the basic stains readily, the lobes being coarsely reticular. The cell body contains many oval granules, which are much coarser than the granules in the polynuclears, and are strongly acidophile. These cells are a little larger than the polynuclears, averaging 10.9 microns in diameter.

With eosin and methylene blue, the nucleus is stained blue and is coarsely reticular. The granules are a bright pinkish red, taking the eosin stain. With Jenner's and Wright's stains, practically the same appearance is presented as with eosin and methylene blue. With Ehrlich's triacid mixture, the nucleus takes a very faint green appearance, while the granules take a deep copper color.

In mast cells the nuclei are similar in shape to those of the polynuclear leucocytes and eosinophiles, but the nucleus takes the stain so faintly that it is often difficult to determine the shape. The cell body remains practically unstained, but contains many coarse granules, which have a strong affinity for basic stains. The granules are either spherical or slightly oblong in shape, and are practically the same size as the granules in the eosinophile cells.

With eosin and methylene blue, both the nucleus and granules are stained blue. The granules take a much deeper stain than the nucleus. With Jenner's stain the granules are deep violet, and with Wright's stain they are deep purple, the nucleus being very faintly stained. With Ehrlich's triacid mixture, the nucleus is stained a very light green, while the granules are stained a blackish green color.

Table I gives the results of the measurements of 100 cells of each variety except the mast cells in which 26 were measured. These measurements were made from the blood of 10 different animals.

TABLE I.—*Measurements of blood cells of the carabao.*

Kind of cell.	Average size.	Maximum size.	Minimum size.
	Microns.	*Microns.*	*Microns.*
Red cell	5.6	5.8	5.3
Lymphocyte	7.3	7.6	7.0
Large mononuclear	10.8	11.2	10.3
Polynuclear leucocyte	9.4	9.6	9.1
Eosinophile	10.9	11.5	10.3
Mast cell	7.8	7.9	7.6

Table II contains the results of numerical determinations of the various cellular constituents of the blood, together with other data regarding properties of the blood.

TABLE II.—*The results of examinations of the blood of 25 supposedly normal carabaos.*

No. of carabao.	Sex.	Red corpuscles per cubic millimeters.	Leucocytes per cubic millimeters.	Hæmoglobin.	Specific gravity.	Relative volume of corpuscles and of plasma.		Time of coagulation.
						Corpuscles.	Plasma.	
				Per cent.				*M. s.*
1	M	5,696,000	8,000	90	1.052	25	75	
2	M	5,400,000	8,000	85	1.052	25	75	
3	M	6,088,000	6,000	85	1.054	28	72	3 10
4	M	5,336,000	8,000	90	1.052	25	75	3 10
5	M	6,272,000	12,000	85	1.054	30	70	3 20
6	M	5,920,000	9,500	85	1.052	26	74	3 25
7	M	6,480,000	16,000	100	1.055	28	72	3 25
8	M	7,000,000	18,500	100	1.056	43	57	3 5
9	M	6,072,000	15,000	95	1.054	35	65	3 20
10	F	5,228,000	6,000	95	1.052	26	74	3 15
11	F	6,192,000	8,000	95	1.054			
12	F	5,296,000	8,000	85	1.052			
13	M	6,760,000	12,000	95				
14	M	6,720,000	8,000	90				
15	M	6,190,000	8,500	85				
16	F	6,210,000	9,250	85				
17	M	6,208,000	10,225	100				
18	M	5,888,000	9,750	90				
19	F	5,392,000	10,000	100				
20	M	6,368,000	14,000	95				
21	M	5,864,000	14,750	100				
22	M	6,512,000	12,000	100				
23	F	5,972,000	8,750	90				
24	M	6,260,000	9,500	95				
25	M	6,108,000	10,000	100				
Average		6,057,520	10,389	92.6	1.0532	29.1	70.9	3 16.2
Maximum		7,000,000	18,500	100	1.056	43	75	3 25
Minimum		5,228,000	6,000	85	1.052	25	57	3 5

It will be noticed from Table II that the highest number of red cells in the counts was 7,000,000 per cubic millimeter. The lowest number was 5,228,000 per cubic millimeter, and the average for the 25 examinations was 6,057,520 cells per cubic millimeter.

The lowest hæmoglobin percentage was 85, the highest percentage 100, and the average for the 25 examinations was 92.6 per cent.

The highest number of leucocytes in the counts was 18,500 per cubic millimeter, and the lowest number 6,000 per cubic millimeter. The average for the 25 examinations was 10,389 per cubic millimeter.

The highest specific gravity was 1.056, the lowest 1.052, and the average for 12 examinations was 1.0532.

In reference to the relative volumes of corpuscles and of plasma, the highest percentage of corpuscles was 43, and the highest percentage of plasma was 75. The lowest percentage of corpuscles was 25, and of plasma 57. The average for 10 examinations gave 29.1 per cent of corpuscles and 70.9 per cent of plasma.

In working out the time of coagulation of the blood, it was found that three minutes and twenty-five seconds was the longest time and three minutes and five seconds the shortest time for coagulation to take place. The average for 8 examinations was three minutes and sixteen seconds plus.

From the table it will be noticed that No. 8 has the highest red-cell count, the highest leucocyte count, 100 per cent hæmoglobin, the highest specific gravity, the largest volume of corpuscles to the smallest volume of plasma, and it took the shortest time for the blood to coagulate.

Table III gives a detailed study of the leucocytes. The number per cubic millimeter and the percentage of each are given. These are based on counts of from 800 to 1,200 cells in each case.

The lymphocytes vary from 10,725 to 2,280 per cubic millimeter, and from 71.5 to 36.9 per cent.

The large mononuclears vary from 1,136 to 240 per cubic millimeter, and from 3 to 10.2 per cent.

The polynuclears vary from 1,648 to 6,290 per cubic millimeter, and from 20.6 to 51.9 per cent.

The eosinophiles vary from 0 to 2.522 per cent. Animal 12 showed no eosinophiles in a count of 872 cells. The percentage of these cells varied between 0 to 23.7.

TABLE III.—*Differential counts of leucocytes in carabao blood.*

No.	Leucocytes.	Lymphocytes.		Large mononuclears.		Polynuclears.		Eosinophilis.		Mast cells.	
		Number.	Percent.	Number.	Percent.	Number.	Percent.	Number.	Percent.	Number.	Percent.
1	8,000	2,984	37.3	312	3.9	3,424	42.8	1,136	14.2	144	1.8
2	8,000	5,056	63.2	816	10.2	1,648	20.6	472	5.9	8	0.1
3	6,000	2,880	48.0	252	4.2	1,740	29.0	960	16.0	168	2.8
4	8,000	3,568	44.6	240	3.0	2,240	28.0	1,896	23.7	56	0.7
5	12,000	4,428	36.9	468	3.9	6,228	51.9	840	7.0	36	0.3
6	9,500	4,370	46.0	466	4.9	3,629	38.2	940	9.9	95	1.0
7	16,000	6,624	41.4	1,136	7.1	5,478	34.3	2,522	15.7	240	1.5
8	18,500	8,880	48.0	925	5.0	6,290	34.0	2,220	12.0	185	1.0
9	15,000	10,725	71.5	735	4.9	3,375	22.5	165	1.1	------	------
10	6,000	3,018	50.3	282	4.7	1,902	31.7	726	12.1	72	1.2
11	8,000	4,216	52.7	320	4.0	2,480	31.0	968	12.1	16	0.2
12	8,000	3,920	49.0	256	3.2	3,520	44.0	------	------	304	3.8
13	12,000	4,992	41.6	564	4.7	4,284	35.7	1,992	16.6	168	1.4
14	8,000	3,472	43.4	432	5.4	2,696	33.7	1,304	16.3	96	1.2
15	8,500	4,020	47.3	332	3.9	2,779	32.7	1,300	15.3	68	0.8
16	9,250	4,542	49.1	388	4.2	3,089	33.4	1,138	12.3	92	1.0
17	10,225	5,716	55.8	389	3.9	3,313	32.4	654	6.4	153	1.5
18	9,750	4,280	43.9	468	4.8	3,919	40.2	946	9.7	137	1.4
19	10,000	4,610	46.1	420	4.2	3,360	33.6	1,520	15.2	90	0.9
20	14,000	6,832	48.8	546	3.9	5,530	39.5	938	6.7	154	1.1
21	14,750	7,847	53.2	605	4.1	5,089	34.5	1,062	7.2	147	1.0
22	12,000	5,664	47.2	516	4.3	3,816	31.8	1,860	15.5	144	1.2
23	8,750	4,235	48.4	394	4.5	3,036	34.7	1,006	11.5	79	0.9
24	9,500	4,474	47.1	371	3.9	3,429	36.1	1,121	11.8	105	1.1
25	10,000	4,880	48.8	460	4.6	3,670	36.7	870	8.7	120	1.2
Average	10,389	5,049	48.5	484	4.6	3,589	34.5	1,142	11.5	115	1.2
Maximum	18,500	10,725	71.5	1,136	10.2	6,290	51.9	2,522	23.7	304	3.8
Minimum	6,000	2,880	36.9	240	3.0	1,648	20.6	0	0	0	0

The mast cells varied from 0 to 304 per cubic millimeter, and from 0 to 3.8 per cent. In animal 9 no mast cells were found in a count of 920 cells.

In averaging the numbers of each kind of cell when fractions of cells were encountered, every fraction below 0.5 was dropped, and each fraction over 0.5 was counted as 1.

SUMMARY

1. In the circulating blood of supposedly normal carabaos over 2 years old the red corpuscles were found to average 6,057,520 per cubic millimeter.

2. The average percentage of hæmoglobin was 92.6.

3. The average number of leucocytes was 10.389 per cubic millimeter.

4. The average specific gravity found was 1.0532.

5. The relative volume of corpuscles to plasma was found to be 29.1 per cent of corpuscles to 70.9 per cent of plasma.

6. The average time for complete coagulation of the blood was found to be three minutes and sixteen seconds plus.

7. The following five varieties of leucocytes were found in the peripheral blood:

(*a*) Lymphocytes. Average size, 7.3 microns in diameter; average number per cubic millimeter, 5.049. They comprise 48.5 per cent of all leucocytes.

(*b*) Large mononuclears. Average diameter, 10.8 microns; average number per cubic millimeter, 484. They comprise 4.6 per cent of all leucocytes.

(*c*) Polynuclears. Average diameter, 9.4 microns. Average number, 3,598 per cubic millimeter. They comprise 34.5 per cent of all leucocytes.

(*d*) Eosinophiles. Average diameter, 10.9 microns. Average number, 1,142 per cubic millimeter. They comprise 11.5 per cent of all leucocytes.

(*e*) Mast cells. Average diameter, 7.8 microns. Average number, 115 per cubic millimeter. They comprise 1.2 per cent of all leucocytes.